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PREFACE

Distinguished Scientists,

We are pleased to encounter the multitude of experts who have made valuable contributions to the scientific area and literature included inside this collection. We express our gratitude to the exceptional researchers who made valuable contributions to our effort.

Over time, substantial progress has been achieved in improving the quality of life and overall welfare of human beings. Notable advancements in recent years have impeded the ability to maintain sustainable living conditions and agricultural practices. Scientists and chapter writers have made substantial contributions to the topics at hand. It is essential for humanity to protect the ecology from more significant dangers. Minor fluctuations in the climate may have a substantial influence on the life cycles of creatures, as well as on the general production of the scientific age. This book explores several themes and proposes answers that are influenced by variables such as population expansion, changing eating preferences, technical improvements in industry, urbanization, climate change, and chemical disasters.

The main objective of this book is to address contemporary scientific issues and offer remedies for challenges in natural resources, agriculture and life sciences. These challenges are predominantly influenced by population growth, shifting dietary patterns, industrial advancement, urbanization, climate change, and chemical disasters.

This book has 22 chapters authored by various writers. As editors, we express our gratitude to all the authors who have made substantial contributions to our readers by sharing their expertise, experience, and ideas. This book aims to enhance understanding about novel scientific technology, the advantages they provide to humanity, and diverse ecosystems via ongoing and upcoming scientific research.

Sincerely Yours,
July, 2024
Prof. Dr. Ahmet KAZANKAYA
Assist. Prof. Dr. Mevlüde Alev ATEŞ

CHAPTER 1

INGREDIENTS AND HEALTH BENEFITS OF WALNUT

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INTRODUCTION

Walnut is a type of fruit that has been consumed globally for many years and it is also an important source of commercial income for many countries. The walnut, which belongs to the species *Juglans regia* L., is cultivated especially in regions such as Anatolia and Iran and has wide usage worldwide (Zhao et al., 2017; Demir et al., 2018). Turkey holds a significant position in walnut production and is a country with a deep-rooted tradition in horticulture. Its geographical location makes Turkey the homeland of many fruit species, making it a rich resource in terms of biodiversity (Ercişli et al., 2012). The world's walnut production is around 3.5 million tons. Turkey, which has an important position in walnut cultivation, ranks fourth in the world walnut production after China, the USA, and Iran. Turkey contributes 9.28% of the world's production with an annual output of 325,000 tons (Nadeem, 2021).

In recent years, there has been increasing interest in natural foods, and within this trend, fruits that are natural sources of antioxidants have come to the fore. Walnut is a food that stands out with its rich nutritional content and popularity. With the growing awareness of the importance of healthy eating and natural foods, plant-based foods, especially nuts like walnuts, are increasingly attracting attention due to their positive effects on human health (Qamar et al., 2020).

Nutritionists and researchers emphasize that walnuts are an important source for health. Walnuts contain nutrients such as protein, healthy fats, minerals, and vitamins. In particular, walnuts are a rich source of protein and contain many of the essential amino acids required by the body. Additionally, walnuts are rich in healthy fats, especially unsaturated fatty acids, which are beneficial for heart health. In terms of minerals, walnuts contain manganese, copper, magnesium, and phosphorus. Walnuts, which are also rich in vitamins, are an important source of vitamin E and B group vitamins. With the combination of these nutritional components, walnuts support a healthy nutritional regime when consumed regularly (Martínez et al., 2010).

Epidemiological and clinical studies indicate that regular walnut consumption can have various positive effects on health. These studies reveal the potential benefits of walnuts in the treatment and prevention of common health issues such as cancer, obesity, diabetes, cardiovascular diseases, and other chronic disorders. For example, the antioxidant properties associated with walnut consumption can help inhibit the growth of cancer cells and reduce inflammation. Moreover, as a fiber-rich food, walnuts can assist in managing metabolic diseases like obesity and diabetes. Furthermore, the high antioxidant content of walnuts can prevent the development of cardiovascular diseases and support heart health. Therefore, these epidemiological and clinical studies demonstrate that regular walnut consumption can play a significant role in overall health (Berkey et al., 2012; Crovesy et al., 2021; Anwar et al., 2020; de Souza et al., 2017).

Walnut is a significant source in terms of antioxidant potential, and this feature is associated with antioxidant phenolic compounds. Antioxidant phenolic compounds fight free radicals by preventing cellular damage and can reduce the effects of oxidative stress. These compounds can prevent the development of various diseases and provide positive effects on overall health. Additionally, the richness of walnuts in organic acids enhances their antioxidant properties and encourages their use for pharmacological purposes. The antioxidant properties of organic acids can be effective in reducing cellular oxidative stress and can play a protective role in health. Moreover, the high content of unsaturated fatty acids in walnuts, particularly oleic acid, linoleic acid, and linolenic acid, is also considered an important source in terms of nutrition. These fatty acids can support cardiovascular health, improve blood lipid profiles, and reduce inflammation. Thus, the antioxidant and fatty acid content of walnuts can provide positive effects on health in various ways (Cosmulescu et al., 2015; Ürün et al., 2021).

Walnut attracts attention with its long history of use not only in nutrition, but also in traditional folk medicine and in the cosmetic and pharmaceutical industries. The reasons behind the use of walnuts in these areas are the health effects of the various compounds it contains. These compounds can generally be divided into various subcategories such as phenolic acids, saponins, quinones, alkaloids, flavonoids, butyl phenyl ether, coumarin, tannic acid and polysaccharides. Phenolic acids have antioxidant properties and can reduce cellular damage. Saponins may exhibit anti-inflammatory and anti-carcinogenic properties. Quinones may have antimicrobial activity, while alkaloids may have a variety of pharmacological effects. Flavonoids may have antioxidant and anti-inflammatory properties. Butyl phenyl ether may exhibit antioxidant and antimicrobial activity. Coumarin may prevent blood clotting and have antioxidant effects. Tannic acid may have anti-inflammatory and antiviral properties, while polysaccharides may support the immune system. The fact that these compounds have various pharmacological and cosmetic effects has supported the use of walnuts in traditional medicine and cosmetic products (Cardoso et al., 2012; Caldas et al., 2012).

Walnut is a fruit with significant importance in human nutrition and provides numerous health benefits. Research indicates that walnuts can play a potential protective role in the treatment and prevention of various diseases. Therefore, the nutritional importance and health benefits of walnuts require increasing research and attention.

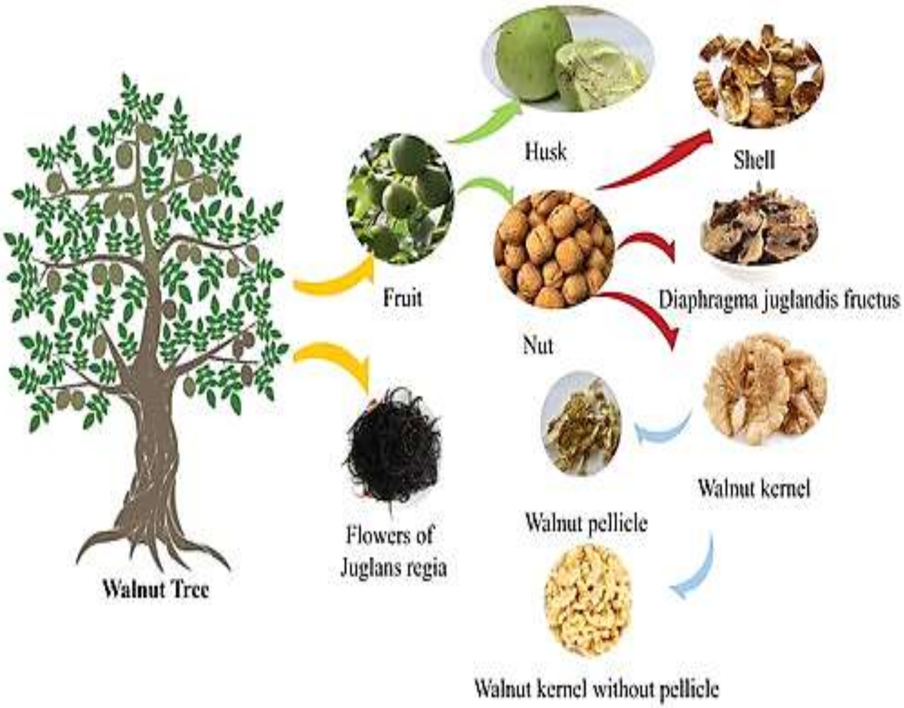

Figure 1. Schematic representation of different parts of the walnut (Ni et al., 2022)

SCIENTIFIC STUDIES ON DIFFERENT COMPONENTS OF THE WALNUT TREE

Recent research has revealed that walnuts play a significant role in human health and nutrition. It is indicated that this fruit is beneficial in the treatment of various diseases such as cardiovascular diseases, certain types of cancer, nervous system health, diabetes, and obesity (Guasch-Ferre et al., 2013; Hardman, 2014; Sánchez-González et al., 2016; Poulose et al., 2014; Pribis, 2016; Cole & Florez, 2020; Njike et al., 2015; Zileli et al., 2016).

Studies show that the antioxidant potential of walnuts is significant and that they are a rich source of phenolic compounds (Cosmulescu et al., 2015). Furthermore, the organic acids found in walnuts are noted for their antioxidant properties, making them widely used for pharmacological purposes (Ürün et al., 2021).

Walnuts typically contain high amounts of unsaturated fatty acids. Particularly rich in oleic, linoleic, and linolenic acids, walnut oil is found to have the highest levels of total polyunsaturated fatty acids and linoleic acid among nuts (Arcan et al., 2021; Okatan et al., 2022; Jahanban-Esfahlan et al., 2019; Kafkas et al., 2020).

Walnuts have been widely used not only as a healthy food but also in traditional folk medicine, and the cosmetics and pharmaceutical industries (Cardoso et al., 2012). The compounds found in walnuts are generally categorized into various subcategories such as phenolic acids, saponins, quinones, alkaloids, flavonoids, butyl phenyl ether, coumarin, tannic acid, and polysaccharides (Caldas et al., 2012).

Phenolic compounds are considered the main antioxidants in walnuts and are associated with anti-carcinogenic, anti-inflammatory, antibacterial, anti-aging, anti-fungal, neuroprotective, and other functions. It has been suggested that treatment with phenolic compounds can improve the endogenous antioxidant system to prevent skin aging (Rahmani et al., 2018).

Walnuts also contain omega-3 fatty acids, particularly alpha-linolenic acid (ALA), and about 6-7% dietary fiber. Moreover, it is noted that walnut consumption can cause changes in the gut microbiome, and different bacterial communities have been linked to many chronic diseases such as cancer, heart disease, and brain health (Emili et al., 2020). Nakanishi and colleagues (2016) have shown that a diet containing walnuts can help prevent colon cancer, and this effect is associated with changes in the gut microbiome.

Walnuts have been an important food item in human nutrition for many years and are recognized as an important plant worldwide due to their rich phytochemical content (Gandev, 2007). The high protein and fat content of walnuts make them indispensable for human nutrition. Additionally, walnuts are rich in protein, vitamins, fats, and minerals and are considered an effective source of phytochemicals such as polyphenols, phenolic acids, and flavonoids (AbuTaha and Wadaan, 2011).

Nutritional value and content are influenced by genotypes, varieties, ecological factors, and soil properties. Research shows that the fats in walnut kernels range from 45-68% and proteins from 13-16% (Martinez et al., 2010; Muradoğlu et al., 2010; Gupta et al., 2019). Therefore, walnuts have played an important role in the functional food market for a long time.

Walnuts are of great scientific interest due to their many health benefits. Various studies have highlighted the physiological importance of the nutrients (such as polyphenols and vitamins) found in the leaves, flowers, shells, and kernels of walnuts (Ni et al., 2021). These studies have focused on the potential protective role of walnut consumption against various diseases such as cancer, intestinal dysbiosis, cardiovascular, and neurological diseases.

The therapeutic effects of a walnut-enriched diet on chronic diseases are particularly associated with its antioxidative and anti-inflammatory activities. Therefore, the synergistic or individual effects of walnut components may play a protective role against various health problems (Ni et al., 2021). As noted by Ni and colleagues (2021), the lipid profile of walnuts is of great interest due to its broad range of biological advantages and functions, contributing to overall health.

Walnut Kernel and Health Effects

The walnut kernel is a significant component of the walnut fruit, making up about half of the fruit's total weight. This content is rich in protein, lipids, and minerals, making it an excellent energy source. Additionally, it stands out for its high B-6 vitamin content and contains significant amounts of B group vitamins. These components are critical for brain health as they enhance memory and support longevity. Furthermore, they help protect heart health and prevent bone loss (Sharma et al., 2022).

Antidiabetic and Hepatoprotective Activities

Aqueous extracts obtained from the walnut fruit have been found to be effective against gram-positive and gram-negative bacterial strains (Poyrazolu and Biyik, 2010; Deshpande et al., 2011). The use of natural antimicrobial compounds in food preservatives is preferred to avoid the potential side effects of chemical preservatives and to reduce antibiotic resistance. Therefore, extracts from various parts of the walnut plant and preparations containing juglone are used in the pharmaceutical field against microbial infections (Khattak et al., 2022).

In a study on the antidiabetic activity of walnuts, it was found that walnuts have a rich polyphenol content that shows strong inhibition against different enzymes such as amylase, maltase, sucrase, and glucosidase. Polyphenolic compounds such as Tellimagradin I, Tellimagradin II, and Casuarictin have also been reported to exhibit antidiabetic activity. These compounds have been observed to have a reducing effect on triglycerides and urinary peroxidase in cases where Type II diabetes is genetically inherited. These findings suggest that walnuts could be used as a potential treatment or preventive agent against metabolic disorders related to diabetes (Fukuda et al., 2004; Jelodar et al., 2007; Jafari et al., 2013).

In a study conducted to observe the protective effect of walnuts on liver health, the hepatoprotective activity was emphasized. It was reported that oral administration of walnut polyphenols showed a protective effect against liver damage in a model of CCL4-induced liver injury in mice. This finding suggests that walnut polyphenols may be a more effective hepatoprotective agent than the commonly used compound curcumin. The essential phytobiotics found in various parts of the walnut were identified as polyphenolic components, which are responsible for oxidative stress-induced liver damage. One of these polyphenolic components, Tellimagrandins I, has been identified as key components of hepatoprotective activity (Hiroshi et al., 2008; Christopoulos and Tsantili, 2011; Choudhary et al., 2020).

Anticancer and Antioxidant Effects

Various researchers have conducted studies on the anticancer activity of walnuts and walnut components such as juglone. Juglone functions as a promising chemopreventive agent against many cancerous cells. It has been reported to have strong cytotoxic effects on human tumor cells, induce apoptosis, and inhibit the growth of sarcoma cells. Additionally, walnut leaves, tree bark, green husk, and other walnut components exhibit significant anticancer properties through mechanisms such as growth inhibition, antiproliferative effects, activation of the mitochondrial death pathway, and the formation of reactive oxygen species (ROS). The molecular foundations of juglone's effects on cell cycle arrest and apoptosis in human endometrial cancer cells have been investigated. Juglone was observed to affect the mitochondrial pathway by increasing ROS levels, thereby triggering apoptosis and preventing the migration of cancer cells. By inducing programmed cell death in endometrial cancer cells, juglone is proposed as a therapeutic strategy for the treatment and prevention of endometrial cancer. These findings suggest that juglone could be a potent natural anticancer agent and warrant further investigation through in vitro studies (Avanzato, 2010; Ji et al., 2011; Bennacer and Cherif, 2016; Al-Snafi, 2018; Arya et al., 2020; Zhang et al., 2019, 2021).

Antioxidant Properties and Effects on the Immune System

Walnut extracts obtained from various parts of the walnut tree have shown antioxidant capacity through free radical scavenging methods and have reduced lipid oxidation (Carvalho et al., 2010; Abbasi et al., 2010; Qamar and Sultana, 2011). This antioxidant activity has been observed against DPPH, H2O2, and superoxide anion radicals. Walnuts are known as a food that supports heart health due to their phytochemicals, lipid-soluble bioactives, and other antioxidants. Because of these properties, walnuts are considered an ideal choice for snacks and dietary supplements. Moreover, consuming walnuts is suggested to improve cognitive function by reducing oxidative stress and enhancing antioxidant defense, thereby potentially reducing the risk of various diseases (Ni et al., 2021).

Research has reported that walnuts enhance memory and learning abilities, acting as a memory enhancer by increasing serotonin levels in the brain and improving memory and learning capacity (Asadi-Shekaari et al., 2013). Another study indicated that polyphenol extracts from green husks and walnut kernels remarkably enhanced learning and memory function in the brain (Shi, 2014).

Walnut fruits have been reported to enhance and strengthen the immune system by increasing phagocytosis in various macrophages and enhancing lymphocyte proliferation (Delaviz et al., 2017; Danh et al., 2020). Therefore, due to its immune-boosting effects, walnuts could be considered as one of the significant medicinal plants used in COVID-19 viral infections.

Leaves and Health Effects

Walnut green leaves are used in the treatment of various health issues. Particularly, they are known to be effective in treating skin disorders, eye irritations, eye pain, and conjunctivitis. They are also used to alleviate appetite loss. An infusion made from the leaves is used for eye wash to alleviate irritations and treat conjunctivitis, and the same tea is known to help heal wounds, acne, and skin allergies (Sharma et al., 2022). It has been found that walnut leaf extract contains antiviral compounds, and Juglone exhibits antiviral properties due to its protein-ligand binding affinity against viruses using virtual docking In Silico methods (Akram et al., 2013; Rosaria et al., 2019).

Furthermore, research has examined the antifungal activities of extracts obtained from walnut leaves. Bioactive compounds such as juglone and eugenol were isolated from leaf extracts using column chromatography. These compounds have been reported to exhibit antifungal properties against various fungal pathogens.

The antifungal activity of walnut leaves against Candida albicans pathogenic isolates was evaluated using different extracts, with methanolic extract showing the maximum antifungal activity (Oliveira et al., 2008). Walnut leaf, bark, fruit, and green husk aqueous extracts have been found effective against both gram-positive and gram-negative bacterial strains (Poyrazolu and Biyik, 2010; Deshpande et al., 2011). Additionally, extracts from walnut leaves have shown microbicidal activity against microorganisms; for instance, leaf extract has been found effective against bacteria causing acne on the skin (Qadan et al., 2005).

Walnut Shell and Health Effects

Walnut shell powder is one of the key components used in beauty products for treating sunburn and sunburn-related issues (Sharma et al., 2022). Various types of extracts such as methanolic, chloroform, acetone, and ethyl acetate extracts obtained from walnut shells have exhibited antifungal properties against different fungal species including Candida, Fusarium solani, Alternaria alternata, and Aspergillus niger infections (Fang et al., 2015; Mohammed et al., 2018). Aqueous extracts derived from walnut green husks have been found effective against both gram-positive and gram-negative bacterial strains (Poyrazolu and Biyik, 2010; Deshpande et al., 2011). Extracts from walnut shells have also demonstrated microbicidal activity against microorganisms (Qadan et al., 2005).

A study highlighted significant activity of acetone extract from walnut shells against Eicinia feotida organisms. Moreover, ethanol, methanol, and benzene extracts from the shells have been reported to exhibit significant anthelmintic (worm-killing) activity against Pheretima posthuma organisms. Researchers have noted that this anthelmintic activity is maximum in the shell's body (Upadhyay et al., 2010).

Walnut inner shell membrane of walnut and health effects

The inner shell of walnut can be used to make both decoctions and tinctures. Decoction can be employed to treat constipation and aid weak digestion, stimulate the liver, and even treat skin disorders (Sharma et al., 2022). Aqueous extracts obtained from the fruit shell have been found effective against both gram-positive and gram-negative bacterial strains (Poyrazolu and Biyik, 2010; Deshpande et al., 2011).

Research on the potential effects of extracts from walnut shells on helminth infections found that acetone extract from walnut shells showed significant activity against Eicinia feotida. In addition, ethanol, methanol, and benzene extracts from the shell were reported to exhibit significant anthelmintic (worm-killing) activity against Pheretima posthumana. Researchers noted that this anthelmintic activity was maximum in the shell's bark (Upadhyay et al., 2010).

CLASSIFICATION OF WALNUT COMPOUNDS

Studies have shown that edible walnuts can provide various health benefits, primarily due to phytochemicals such as phenolic compounds. Flavonoids derived from phenolic substances, phenolic acids, tannins, and naphthoquinones fall into the category of plant polyphenols, ranking second only to cellulose, hemicellulose, and lignin in plants (Şen et al., 2006). These compounds regulate plant growth and development, contribute to metabolic reactions, and can have positive effects on health. Particularly, walnut polyphenols are one of the biologically active components of walnuts and offer beneficial health effects. Flavonoids can form glycosides and aglycones in plants, while phenolic acids belong to the class of organic acids, with gallic acid being one of the most common components (Beiki et al., 2018).

Tannins are divided into two main groups: hydrolyzable tannins and ellagitannins, which include gallotannins. Ellagitannins contain gallic acid and hexahydroxydiphenic acid (HHDP) and are generally more stable compared to gallotannins. Quinones are molecules typically characterized by unsaturated structures and hold particular importance in traditional Chinese medicine (Beiki et al., 2018; Nieto et al., 2020).

Walnuts have been a significant food source in human nutrition for many years. They are recognized globally as an important plant due to their rich phytochemical content (Gandev, 2007). Different components have been observed to serve different functions, and these components may vary in their proportions in different parts of the walnut. For example, while walnut husks significantly contribute to the tannin content of walnuts, the inner kernel is rich not only in tannins but also in high protein and fat content, making walnuts indispensable in human nutrition. Walnuts are a rich source of protein, vitamins, fats, minerals, and effective phytochemicals (Table 1; Figure 2) (AbuTaha and Wadaan, 2011; Alasalvar and Shahidi, 2020).

Walnut is a significant food source in daily nutrition, providing various nutrients (Table 1). Considering a daily energy requirement of approximately 8400 kJ, consuming 100 grams of walnuts meets about 32% of this need. Similarly, while the recommended daily fat intake ranges between 20-35%, consuming 100 grams of walnuts covers 50% of this requirement. Rich in protein as well, walnuts provide about 30% of the daily protein requirement.

Table 1 Nutritional content value of various constituents in walnut

Principle compounds	Values per 100 g	Principle compounds	Values per 100 g
Energy	2738 kJ	Fiber	6.4 g
Fat	65.2 g	Folate	98 µg
Protein	15.2 g	Plant sterols	72 mg
Vitamins		**Minerals**	
Vitamin A	20 mg	Aluminum	0.58 mg
Vitamin C	1.3 mg	Phosphorus	346 mg
Vitamin E	20.83 mg	Calcium	98 mg
Vitamin K	207 mg	Potassium	441 mg
Folates (B9 vitamins)	98 mg	Magnesium	158 mg
Niacin (B3 vitamins)	1.125 mg	Sodium	2 mg
Pantothenic acid	0.570 mg	Iron	2.9 mg

Pyridoxine	0.537 mg	Copper	1.5 mg
Riboflavin (B2 vitamins)	0.150 mg	Manganese	3.8 mg
Thiamin (B1 vitamins)	0.541 mg	Zinc	3.09 mg
Fatty Acids			
Unsaturated Fatty Acids		**Saturated Fatty Acids**	
Palmitoleic acid C16:1	0.77	Myristic acid C14:0	0.24
Oleic acid C18:1	25.26	Palmitic acid C16:0	4.28
Gadoleic acid C20:1	0.05	Stearic acid C18:0	1.85
Total MUFA	22.37	Arachidic acid C20:0	0.19
Linoleic acid C18:2	57.10	Total SFA	7.21
Linolenic acid C18:3	10.34	**PUFA/SFA**	9.91
Total PUFA	4.29		

Source: Raja et al. (2012), Gajendra and Sharique (2016), Al-Snafi (2018), Verma and Sharma (2020); http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl

Walnuts are also rich in minerals such as iron and zinc. For instance, consuming 100 grams of walnuts fulfills 32% of the daily iron requirement and 28% of the zinc requirement. Being rich in omega-3 fatty acids, walnuts can contribute significantly to weekly omega-3 intake. Among the vitamins found in nutrient-rich walnuts, vitamin A provides 2.3% of the daily requirement, vitamin E offers 139.2%, and vitamin K provides 207%. Additionally, B vitamins including folate, niacin, pantothenic acid, pyridoxine, riboflavin, and thiamine in walnuts contribute to a significant portion of the daily requirement.

Walnuts are notable for their high fiber content, providing 16.2% of the daily fiber intake. Moreover, walnuts meet 9.8% of the daily calcium requirement, 9.4% of potassium, and 37.6% of magnesium. As a prominent food among oily seeds, walnuts boast the highest content of omega-3 and omega-6 fatty acids. Beyond its lipid content, walnuts are recognized for supporting brain health and development, their rich protein content, and essential compounds vital for the body. Therefore, walnuts are considered a highly important functional food for promoting healthy nutrition and overall health (Şen et al., 2006).

Fatty acids are among the key components determining the functional properties of walnuts. According to data from Table 1, the majority of walnut oil consists of polyunsaturated fatty acids (72%), followed by monounsaturated fatty acids (18%) and saturated fatty acids (10%). Additionally, vitamin E and other antioxidants present in walnuts (such as phytosterols and polyphenols) contribute to its recognition as a functional food. These compounds are known to play a protective role against cardiovascular diseases, certain types of cancer, and the negative effects of aging. For example, vitamin E prevents LDL cholesterol oxidation, thereby reducing the risk of heart disease, and polyphenols in walnuts are noted for strengthening the immune system. Clinical studies support the role of walnut consumption in reducing the risk of cardiovascular diseases and cancer (Anderson, 2001).

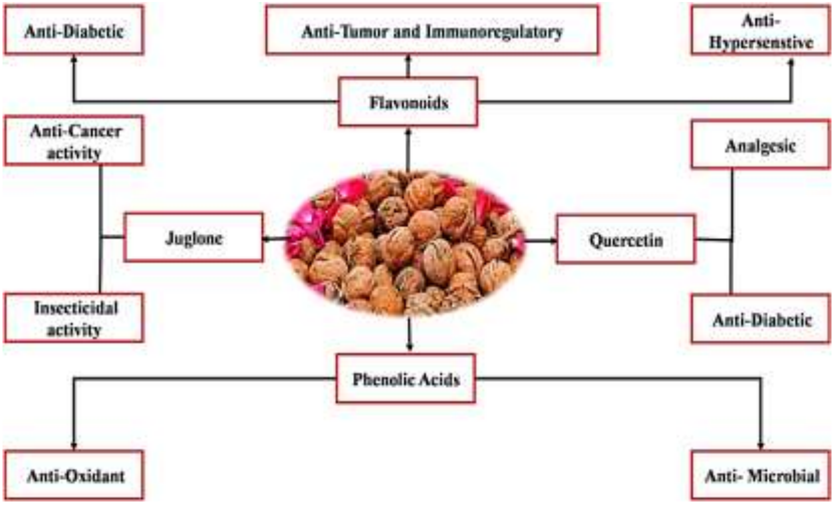

Figure 2. Dominant phytoconstituents and their pharmacological properties of walnut (Sharma et al., 2022)

WALNUT AS A FUNCTIONAL FOOD AND ANATOLIAN CULTURE

Functional foods are foods that go beyond basic nutrients and provide specific health benefits. These foods contain special components that can support body functions or prevent certain health problems. Functional foods are generally rich in nutrients such as vitamins, minerals, antioxidants, probiotics, prebiotics, and fatty acids, which have positive effects on human health (Alaşalvar and Pelvan, 2009).

Approximately 2500 years ago, the famous saying of the medical pioneer Hippocrates, "Let food be thy medicine and medicine be thy food," still holds importance today. Among functional foods recognized for their positive health effects, antioxidants are one of the most common. Antioxidants are derived from natural sources such as phenolic compounds and are frequently used in functional food products. Research reports indicate that these compounds can lower cholesterol levels in the blood, provide protective effects against osteoporosis and cancer, and may also be effective in preventing unwanted bacterial infections. Therefore, antioxidants are highly valuable components for health (Şen et al., 2006; Tekeli et al., 2008).

Walnuts hold a significant place in Turkish cuisine and are used in many dishes. This usage plays a critical role in shaping traditional Turkish flavors and becoming part of our cultural heritage. Walnut kernels are considered a key ingredient in various Turkish dishes and sweets (Şen, 2005).

In Turkish cuisine, walnuts are used across a wide range of dishes, from salads to pastries, cakes to desserts. Particularly in salads, walnuts are known to enhance both flavor and nutritional value. Walnut-filled pastries and cakes are among the indispensable flavors of Turkish cuisine. Walnuts are also commonly used in Turkish desserts, especially in traditional sweets like baklava, adding both flavor and nutritional value. In addition to meals, walnuts are frequently used in snacks. Consuming walnut kernels either salted or sweetened by removing the shell is quite popular (Şen et al., 2006).

Baklava, especially varieties made with walnut kernels, is one of the most beloved Turkish desserts. Besides baklava, walnuts are essential in sweets such as köme (a type of fruit roll), pestil (fruit leather), walnut jam (walnut pekmez), walnut lokum (Turkish delight), and nuska (walnut-based confection). Walnuts in Turkish cuisine extend beyond desserts. For instance, "bastık," a type of pestil from Kahramanmaraş region filled with walnuts, holds significant culinary importance. Moreover, in some villages of Central Anatolia, "batırak," a dish made with moistened bulgur and walnuts, serves as a substantial component. Villagers rely on traditional knowledge, noting that combining walnuts with bulgur complements protein deficiency, making it an excellent food source. Therefore, walnuts play a crucial role as an important ingredient in traditional recipes like raw köfte (Çiğ köfte) in certain regions (Şen et al., 2006).

REFERENCES

AbuTaha, N., & Wadaan, M. (2011). Utility and importance of walnut, Juglans regia Linn: a review. African Journal of Microbiology Research, 5(32), 5796–5805.

Akram, E., Jalal, Z. M., PejmanM, S. R., & Somayeh, O. (2013). Hepatoprotective effects of Juglans regia extract against CCl4-induced oxidative damage in rats. Pharmaceutical Biology, 51(5), 558–565.

Alasalvar, C., & Shahidi, F. (2010). Tree nuts: Composition, phytochemicals, and health effects. Chromatographia, 72(5-6), 589.

Alaşalvar, C., & Pelvan, E. (2009). Günümüzün ve geleceğin gıdaları fonksiyonel gıdalar. Bilim ve Teknik Dergisi, 8, 26-29.

Al-Snafi, A. E. (2018). Chemical constituents, nutritional, pharmacological and therapeutic importance of Juglans regia - a review. IOSR Journal of Pharmacy, 8(11), 1–21.

Anderson, K. J., Teuber, S. S., Gobeille, A., Cremin, P., Waterhouse, A. L., & Steinberg, F. M. (2001). Walnut Polyphenolics Inhibit invitro Human Plasma and LDL Oxidation. The American Journal of Nutrition, 131, 2837-2842.

Anwar, M., Birch, E. J., Ding, Y., & Bekhit, E. D. (2020). Water-soluble non-starch polysaccharides of root and tuber crops: extraction, characteristics, properties, bioactivities, and applications. Critical Reviews in Food Science and Nutrition. https://doi.org/10.1080/10408398.2020

Arcan, Ü. M., Sütyemez, M., Bükücü, Ş. B., Özcan, A., Gündeşli, M. A., Kafkas, S., & Kafkas, N. E. (2021). Determination of fatty acid and tocopherol contents in Chandler× Kaplan-86 F1 walnut population. Turkish Journal of Agriculture and Forestry, 45, 434–453.

Arcan, Ü.M., Sütyemez, M., Bükücü, Ş.B., Özcan, A., Gündeşli, M.A., Kafkas, S., Kafkas, N.E. (2021). Determination of fatty acid and tocopherol contents in Chandler* Kaplan-86 F1 walnut population. Turkish Journal of Agriculture and Forestry, 45, 434-453.

Arya, A. K., Arora, M., & Singh, F. M. A. (2020). A review on pharmacological activity of Juglans regia. International Journal of Pharmacy, 7(1), 1–11. https://doi.org/10.13040/IJPSR.0975-8232.IJP.7(1).1-11

Avanzato, D. (2010). Traditional and modern uses of walnut. Acta Hortic, 861, 89–96. https://doi.org/10.17660/ActaHortic.2010.861.11

Beiki, T., Najafpour, G. D., & Hosseini, M. (2018). Evaluation of antimicrobial and dyeing properties of walnut (Juglans regia L.) green husk extract for cosmetics. Coloration Technology, 134, 71–81.

Bennacer, A., & Cherif, H. S. (2016). Contribution to the ethnobotanical, phytochemical, antimicrobial and antioxidant study of the leaves aqueous extract of the common walnut Juglans regia L. International Journal of Pharmacology, Phytochemistry and Ethnomedecine, 7, 41–52.

Berkey, C. S., Tamimi, R. M., Willett, W. C., Rosner, B., Hickey, M., Toriola, A. T., Frazier, A. L., & Colditz, G. A. (2020). Adolescent alcohol, nuts, and fiber: combined effects on benign breast disease risk in young women. NPJ Breast Cancer, 6(1), 61. https://doi.org/10.1038/s41523-020-00206-4

Cardoso, B. R., Duarte, G. B. S., Reis, B. Z., & Cozzolino, S. M. F. (2017). Brazil nuts: Nutritional composition, health benefits and safety aspects. Food Research International, 100 (Pt 2), 9–18. doi: 10.1016/j.foodres.2017.08.036.

Christopoulos, M. V., & Tsantili, E. (2011). Effects of temperature and packaging atmosphere on total antioxidants and colour of walnut (Juglans regia L.) kernels during storage. Scientia Horticulturae, 131, 49–57.

Chudhary, Z., Khera, R. A., Hanif, M. A., Ayub, M. A., & Hamrouni, L. (2020). Walnut. In: Medicinal Plants of South Asia. Elesiver, 671–684. https://doi.org/10.1016/B978-0-08-102659-5.00049-5.

Cole, J. B., & Florez, J. C. (2020). Genetics of diabetes mellitus and diabetes complications. Nature Reviews Nephrology, 16, 377–390.

Cole, J.B., & Florez, J.C. (2020). Genetics of diabetes mellitus and diabetes complications. Nature Reviews Nephrology, 16, 377-390.

Cosmulescu, S., Trandafir, I., & Nour, V. (2015). Chemical Composition and Antioxidant Activity of Walnut Pollen Samples. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 43, 361-365.

Cosmulescu, S., Trandafir, I., & Nour, V. (2015). Chemical Composition and Antioxidant Activity of Walnut Pollen Samples. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 43, 361–365.

Crovesy, L., Kaipperf, V. C., Santos, L., Marcelly, C. O., Magno, F. C. C. M., Fialho, E., & Rosado, E. L. (2021). Profile of polyphenols intake by women with different class of obesity: Consumption of these compounds does not reflect healthy eating. Nutrition, 82, 111045. https://doi.org/10.1016/j.nut.2020.111045

Danh, C. V., Trang, H. D., & Nguyenb, T. L. (2020). An overview of phytochemicals and potential health-promoting properties of black walnut. RSC Advances, 10, 33378. https://doi.org/10.1039/d0ra05714b

de Souza, R. G. M., Schincaglia, R. M., Pimentel, G. D., & Mota, J. F. (2017). Nuts and human health outcomes: a systematic review. Nutrition, 9(12), 1311.

Delaviz, H., Mohammadi, J., Ghalamfarsa, G., & Mohammadi, B., Farhadi, N. (2017). A review study on phytochemistry and pharmacology applications of Juglans regia plant. Pharmacognosy Reviews, 11, 145–152.

Demir, B., Sayıncı, B., Çetin, N., Yaman, M., Çömlek, R., Aydın, Y., & Sütyemez, M. (2018). Elliptic Fourier based analysis and multivariate approaches for size and shape distinctions of walnut (Juglans regia L.) cultivars. Grasas y Aceites, 69, e262.

Demir, B., Sayıncı, B., Çetin, N., Yaman, M., Çömlek, R., Aydın, Y., & Sütyemez, M. (2018). Elliptic Fourier based analysis and multivariate approaches for size and shape distinctions of walnut (Juglans regia L.) cultivars. Grasas Aceites, 69, e271.

Deshpande, R. R., Kale, A. R., Ruikar, A. D., Panvalkar, P. S., Kulkarni, A. A., Deshpande, N. R., & Salvekar, J. P. (2011). Antimicrobial activity of different extracts of Juglans Regia L. against oral microflora. International Journal of Pharmaceutical Sciences, 3, 200–201.

Emili, M., Guidi, S., Uguagliati, B., Giacomini, A., Bartesaghi, R., & Stagni, F. (2020). Treatment with the flavonoid 7,8-Dihydroxyflavone: A promising strategy for a constellation of body and brain disorders. Critical Reviews in Food Science and Nutrition. doi: 10.1080/10408398.2020.1810625.

Ercişli, S., Sayıncı, B., Kara, M., Yildiz, C., & Özturk, I. (2012). Determination of size and shape features of walnut (Juglans regia L.) cultivars using image processing. Scientia Horticulturae, 131, 47–55.

Ercişli, S., Sayinci, B., Kara, M., Yildiz, C., & Özturk, I. (2012). Determination of size and shape features of walnut (Juglans regia L.) cultivars using image processing. Scientia Horticulturae, 131, 47–55.

Fang, F., Yingxin, Q., Ling, Q., Qing, F., Liangzhong, Z., Shuang, C., Qiang, L., & Duo, Z. (2015). Juglone exerts antitumor effect in ovarian cancer cells. Iranian Journal of Basic Medical Sciences, 18, 544–548.

Fukuda, T., Ito, H., & Yoshida, T. (2004). Effect of the walnut polyphenol fraction on oxidative stress in type 2 diabetes mice. BioFactors, 2, 251–253.

Gajendra, M., & Sharique, A. (2016). A recent update on the medicinal properties and use of aloe vera in the treatment of various ailments. Bioscience Biotechnology Research Communications, 9(2), 273–288.

Gandev, S. (2007). Budding and grafting of the walnut (Juglans regia L.) and their effectiveness in Bulgaria (review). Bulgarian Journal of Agricultural Science, 13, 683–689.

Guasch-Ferré, M., Bulló, M., Martínez-González, M. Á., Ros, E., Corella, D., Estruch, R., & Salas-Salvadó, J. (2013). Frequency of nut consumption and mortality risk in the PREDIMED nutrition intervention trial. BMC Medicine, 11, 164.

Guasch-Ferre, M., Bulló, M., Martínez-González, M.Â., Ros, E., Corella, D., Estruch, R., & Salas-Salvadó, J. (2013). Frequency of nut consumption and mortality risk in the PREDIMED nutrition intervention trial. BMC Medicine, 11(164).

Gupta, A., Behl, T., & Panichayupakaranan, P. (2019). A review of phytochemistry and pharmacology profile of Juglans regia. Obesity Medicine, 16, 1–7. https://doi.org/10.1016/j.obmed.2019.100142

Hardman, W. E., & Ion, G. (2008). Suppression of implanted MDA-MB 231 human breast cancer growth in nude mice by dietary walnut. Nutrition and Cancer, 60, 666–674.

Hardman, W.E. (2014). Walnuts have potential for cancer prevention and treatment in mice. Journal of Nutrition, 144, 555S-560S.

Hiroshi, S., Tanaka, J., Kikuchi, M., Fukuda, T., Ito, H., Hatano, T., & Yoshida, T. (2008). Walnut polyphenols prevent liver damage induced by carbon tetrachloride and d-galactosamine hepatoprotective hydrolyzable tannins in the kernel pellicles of walnut. Journal of Agricultural and Food Chemistry, 56, 4444–4449.

Jafari, T., Fallah, A. A., & Azadbakht, L. (2013). Role of dietary n-3 polyunsaturated fatty acids in type 2 diabetes: a review of epidemiological and clinical studies. Maturitas, 74, 303–308.

Jahanban-Esfahlan, A., Ostadrahimi, A., Tabibiazar, M., & Amarowicz, R. (2019). A comprehensive review on the chemical constituents and functional uses of walnut (Juglans spp.) husk. International Journal of Molecular Sciences, 20(3920).

Jahanban-Esfahlan, A., Ostadrahimi, A., Tabibiazar, M., & Amarowicz, R. (2019). A comprehensive review on the chemical constituents and functional uses of walnut (Juglans spp.) husk. International Journal of Molecular Sciences, 20, 3920.

Jelodar, G., Mohsen, M., & Shahram, S. (2007). Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan induced diabetic rats. African Journal of Traditional, Complementary, and Alternative Medicines, 4, 299–305.

Ji, Y., HongYuan, Q.Z., & Xiang, Z. (2011). Juglone induced apoptosis in human gastric cancer SGC-7901 cells via the mitochondrial pathway. Experimental and Toxicologic Pathology, 63, 69–78.

Kafkas, E., Attar, S. H., Gundesli, M. A., Ozcan, A., & Ergun, M. (2020). Phenolic and fatty acid profile, and protein content of different walnut cultivars and genotypes (Juglans regia L.) grown in the USA. International Journal of Fruit Science, 20, S1711–S1720.

Kafkas, E., Attar, S.H., Gündeşli, M.A., Ozcan, A., & Ergun, M. (2020). Phenolic and fatty acid profile, and protein content of different walnut cultivars and genotypes (Juglans regia L.) grown in the USA. International Journal of Fruit Science, 20, S1711-S1720.

Khattak, P., Khalil, T. F., Bibi, S., Jabeen, H., Muhammad, N., Khan, M. A., & Liaqat, S. (2022). Juglans regia (walnut tree) bark in dentistry. PBMJ. https://doi.org/10.54393/pbmj.v5i2.201

Martinez, M. L., Labuckas, D. O., & LamarqueAL, M. D. M. (2010). Walnut (Juglans regia L.) genetic resources, chemistry, by-products. Journal of the Science of Food and Agriculture, 90, 1959–1967.

Meral R, Doğan İS, Kanberoğlu GS. (2012). Fonksiyonel Gıda Bileşeni Olarak Antioksidanlar. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 2(2), 45-50.

Mohammed, N., Kasim, H., Hussein, A. S., Emad, A. M., & Hassan, A. M. (2018). Antifungal activity of alcoholic extract of Juglans regia against Phytopathogenic Rhizoctonia solani. Chemical Research Journal, 3(4), 105–109.

Muradoğlu, F., & Balta F. (2007). Hakkari Yöresi Cevizlerinin (Juglans regia L.) Seleksiyon yolu İle Islahı. Türkiye 5. Ulusal Bahçe Bitkileri Kongresi, 4-7 Eylül, Erzurum.

Nadeem, M. A. (2021). Deciphering the genetic diversity and population structure of Turkish bread wheat germplasm using iPBS-retrotransposons markers. Molecular Biology Reports, 48, 6739–6748.

Nadeem, M.A. (2021). Deciphering the genetic diversity and population structure of Turkish bread wheat germplasm using iPBS-retrotransposons markers. Molecular Biology Reports, 48, 6739–6748.

Ni, Z. J., Zhang, Y. G., Chen, S. X., Thakur, K., Wang, S., Zhang, J. G., ... & Wei, Z. J. (2022). Exploration of walnut components and their association with health effects. Critical Reviews in Food Science and Nutrition, 62(19), 5113-5129.

Njike, V. Y., Ayettey, R., Petraro, P., Treu, J. A., & Katz, D. L. (2015). Walnut ingestion in adults at risk for diabetes: Effects on body composition, diet quality, and cardiac risk measures. BMJ Open Diabetes Research & Care, 3, e000115.

Njike, V.Y., Ayettey, R., Petraro, P., Treu, J.A., & Katz, D.L. (2015). Walnut ingestion in adults at risk for diabetes: Effects on body composition, diet quality, and cardiac risk measures. BMJ Open Diabetes Research & Care, 3, e000115.

Okatan, V., Gündeşli, M. A., Kafkas, N. E., & Attar, Ş. H. (2022). Phenolic Compounds, Antioxidant Activity, Fatty Acids and Volatile Profiles of 18 Different Walnut (Juglans regia L.) Cultivars and Genotypes. Erwerbs Obstbau, 64, 247–260.

Oliveira, I., Sousa, A., Ferreira, I. C. F. R., Bento, A., Stevinhol, L. E., & Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green husks. Food and Chemical Toxicology, 46, 2326–2331.

Poulose, S. M., Miller, M. G., & Shukitt-Hale, B. (2014). Role of walnuts in maintaining brain health with age. Journal of Nutrition, 144, 561S–566S.

Poulose, S.M., Miller, M.G., & Shukitt-Hale, B. (2014). Role of walnuts in maintaining brain health with age. Journal of Nutrition, 144, 561S-566S.

Poyrazolu, E. C., & Biyik, H. (2010). Antimicrobial activity of the ethanol extracts of some plants natural growing in Aydin Turkey. African Journal of Microbiology Research, 4, 2318–2323.

Pribis, P. (2016). Effects of Walnut Consumption on Mood in Young Adults-A Randomized Controlled Trial. Nutrients, 8, 668.

Pribis, P. (2016). Effects of Walnut Consumption on Mood in Young Adults-A Randomized Controlled Trial. Nutrients, 8, 668.

Qadan, F., Al-Adham, I. S., & Nahrstedt, A. (2005). Characterization of antimicrobial polymeric procyanidins from Juglans regia leaf extract. European Journal of Scientific Research, 11, 438–443.

Qamar, S., Manrique, Y. J., Parekh, H., & Falconer, J. R. (2020). Nuts, cereals, seeds and legumes proteins derived emulsifiers as a source of plant protein beverages: a review. Critical Reviews in Food Science and Nutrition, 60(16), 2742–2762. https://doi.org/10.1080/10408398.2019.1657062

Rahmani, F., Dehganiasl, M., Heidari, R., Rezaee, R., & Darvishzadeh, R. (2018). Genotype impact on antioxidant potential of hull and kernel in Persian walnut (Juglans regia L.). International Food Research Journal, 25(1), 35-42.

Raja, G., Shaker, I. A., Sailaja, I., Swaminathan, R., & Kondaveeti, S. B. (2012). Nutritional analysis of nuts extract of Juglans regia L. International Journal of Bioassays, 1, 68–73.

Ros, E. (2010). Health benefits of nut consumption. Nutrients, 2(7), 652-682.

Rosaria, A., Floriana, D., Giuseppe, A. M., Simone, R., et al. (2019). Antibacterial and anti-biofilm activities of walnut pellicle extract (Juglans regia L.) against coagulase-negative staphylococci. Natural Product Research, 35, 1–5.

Sánchez-González, C., Ciudad, C. J., Noe, V., & Izquierdo-Pulido, M. (2017). Health benefits of walnut polyphenols: an exploration beyond their lipid profile. Critical Reviews in Food Science and Nutrition, 57(16), 3373-3383.

Sánchez-González, C., Ciudad, C.J., Izquierdo-Pulido, M., & Noe, V. (2016). Urolithin A causes p21 up-regulation in prostate cancer cells. European Journal of Nutrition, 55, 1099-1112.

Sharma, M., Sharma, M., & Sharma, M. (2022). A comprehensive review on ethnobotanical, medicinal and nutritional potential of walnut (Juglans regia L.). Proceedings of the Indian National Science Academy, 88(4), 601-616.

Şen, S.M. (2005). Türkiye'de Cevizin Dünü. Bugünü ve Yarını. Bahçe Ceviz. 34(1):15-17

Şen, S.M., Karadeniz, T., & Beyhan, Ö. (2011). Sorularla Ceviz Yetiştiriciliği. ISBN:978-605-89150-3-9 ÜÇM Yayınları/5 Tarımsal Yayınlar/3. Ankara

Şen, S.M., Kazankaya, A., Yarılgaç, T., & Doğan, A. (2006). Bahçeden Mutfağa Ceviz, 1-234, Maji Yayınları

Tekeli, Y., Sezgin, M., & Şanda, M. A. (2008). Konya'da Yetişen Centaurea Pterocaula Truatvin Fenolik Yapısı ve Antioksidan Etkisi. Süleyman Demirel Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi (E-Dergi), 3(1), 35-41.

Ürün, I., Attar, S. H., Sönmez, D. A., Gündesli, M. A., Ercişli, S., Kafkas, N. E., ... & Duralija, B. (2021). Comparison of polyphenol, sugar, organic acid, volatile compounds, and antioxidant capacity of commercially grown strawberry cultivars in Turkey. Plants, 10, 1654.

Ürün, I., Attar, S.H., Sönmez, D.A., Gündeşli, M.A., Ercişli, S., Kafkas, N.E.,... Duralija, B. (2021). Comparison of polyphenol, sugar, organic acid, volatile compounds, and antioxidant capacity of commercially grown strawberry cultivars in Turkey. Plants, 10(1654).

Verma, G., & Sharma, V. A. (2020). A scientific update on Juglans regia Linn. Asian Journal of Pharmaceutical Research and Development, 8(3), 166–175. https://doi.org/10.22270/ajprd.v8i3.741

Zhang, Y. Y., Ni, Z., Elam, E., Fan, Z., Thakur, K., Wang, S., ... & Wei, Z. J. (2021). Juglone, a novel activator of ferroptosis induces cell death in endometrial carcinoma Ishikawa cells. Food Function. https://doi.org/10.1039/D1FO00790D

Zhang, Y. Y., Zhang, F., Zhang, Y. S., Thakur, K., Zhang, J. G., Liu, Y., ... & Wei, Z. J. (2019). Mechanism of Juglone-induced cell cycle arrest and apoptosis in Ishikawa human endometrial cancer cells. Journal of Agricultural and Food Chemistry, 67(26), 7378–7389. https://doi.org/10.1021/acs.jafc.9b02759

Zhao, H., Bai, H., Jing, Y., Li, W., Yin, S., & Zhou, H. (2017). A pair of taxifolin-3-O-arabinofuranoside isomers from Juglans regia L. Natural Product Research, 31, 945–950.

Zhao, H., Bai, H., Jing, Y., Li, W., Yin, S., & Zhou, H. (2017). A pair of taxifolin-3-O-arabinofuranoside isomers from Juglans regia L. Natural Product Research, 31, 945–950.

Zileli, R., Şemşek, O., Ozkamcı, H., & Gürkan Diker, G. (2016). The Attendance to Sport of Women Living in Bilecik, Obesity Prevalence and Risk Factors. Marmara University Journal of Sports Science, 1, 85-98.

Zileli, R., Şemşek, O., Ozkamcı, H., & Gürkan Diker, G. (2016). The Attendance to Sport of Women Living in Bilecik, Obesity Prevalence and Risk Factors. Marmara University Journal of Sports Science, 1, 85–98.

CHAPTER 2

HAPLOID INDUCTION TECHNIQUES IN PLANTS

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1. INTRODUCTION

The plants with the gametic chromosome number (n) are called as haploid (Kasha and Maluszynski, 2003) . In a diploid (2n) species, the haploid could also be called monoploid (x) because they have only one set of chromosomes. In polyploid species, the haploids (n) have more than one set of chromosomes and are polyhaploids. The haploid plant from an autotetraploid (4x) with four sets of one genome was originally called a dihaploid (because n=2x). When the chromosome number of a haploid is doubled, it should be called a doubled haploid (DH) and not a dihaploid. The dihaploid is not homozygous as it represents two chromosome sets selected from four sets in the autotetraploid, whereas the doubled haploid from a monoploid or an allohaploid should be completely homozygous.

Haploid plants can not produce seed because they are sterile. Therefore chromosome number of haploid plant must be doubled spontaneously or by induction in order to use them in the production or breeding.

2. USING HAPLOID PLANTS IN THE PLANT BREEDING AND GENETICS

Haploid plants could be used in plant breeding and genetics as fllowings;

2.1. In Shortening Breeding Cyle

The induction of haploids and then doubling of their chromosomes by spontaneous or inducing are widely used techniques in advanced breeding programs of several agricultural species. They have been successfully used for commercial cultivar devlopment of species such as asparagus, barley, Brown mustard, eggplant, melon, pepper, rapeseed, rice, tobacco, triticale, wheat and more than 300 varieties have already been released (Kumar and Choudhary, 2020). Completely homozygous plants can be established in one generation by using DH technology, thus saving several generations of selfing comparing to conventional methods, by which completely homozygous plants can not be obtained (Figure 1 and Table 1).

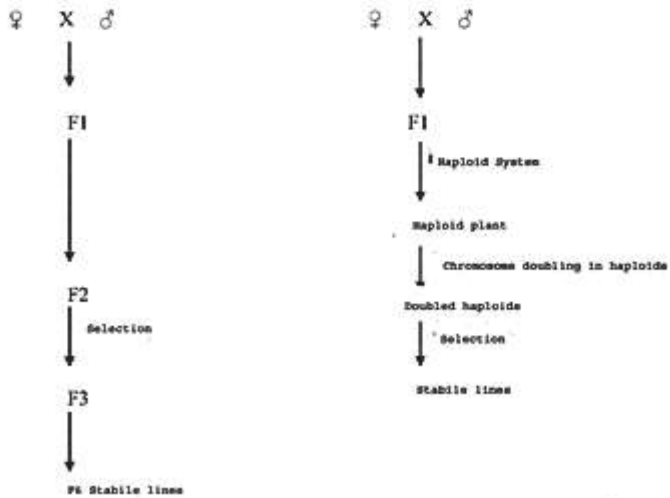

Figure 1. Obtaining homozigot lines by conventional method and haploid doubled haploid technique

Table 1. Difference between DH method and conventional method

Characteristic	DH method	Conventional method
Time required for developing pure line	1 year or 1 crop season	3-5 years
Time required for cultivar development	2-3 years	7-8 years
Fixation of heterosis	possible	Not possible
Expenditures	More than Conventional method	Less than DH method

The role of DH in the breeding process largely depends on the reproduction mode of the plant species. In self-pollinated species, they can represent final cultivars or they can be used as parental lines in hybrid production or testcrosses of cross-pollinated species.

The first variety produced via doubled haploidy was the barley cultivar "Mingo" in the late 1970's in Canada (Thomas, et.al., 2003). Doubled haploidy is now widely used in variety production of many crops . The vast majority of varieties derived from doubled haploidy are in barley (96), followed by rapeseed (47), wheat (20) and the rest.

Back cross breeding is a conventional procedure in transferring a gene of interest particularly of disease resistance usually from a donor genotype to a high yielding but susceptible genotype (recipient) of the same species to make it disease resistant. This is done by crossing donor and recipient parents during 1st year and recurrent back crossing of F_1 hybrid to recipient parent to reconstitute/ restore the genes of high yield in new cultivar. In this way, BC1, BC2, BC3, BC4 and BC5 generations are obtained as a result of back crossing and in BC5, almost 99 % of genes of recipient high yielding parent and gene of disease resistance by selection for disease resistant plants. Hence, it takes 5-6 years to recover the genes of recipient parent. Another problem in back cross breeding is identification of gene of interest or disease resistance at each generation particularly if the disease resistance is controlled by recessive gene. If the gene of our interest is recessive in nature, we have to self each BC generation so that recessive homozygotes are developed from heterozygotes resulted from each back cross. This takes twice time as compared to dominant gene.

Opposite to the problems related with conventional back cross method, DH technology combined with molecular markers not only solves the problems mentioned above but also decreases the variety development period significantly. A molecular marker is used to identify a gene of interest in a back cross generation and DH are developed to get the homozygosity in a single generation (Figure 2) . Hence, double haploidy combined with Marker Assisted Selection (MAS) increases the effectiveness and precision of selection of desirable traits. It is also helpful in stacking resistance genes.

Homozygosity is more important for the plants with very long juvenile phase (period from seed to flowering), such as fruit trees, bulbous plants and forestry trees. Even if repeated self pollination is possible achievement of homozygosity, especially in this group of plants, is an extremely long process.

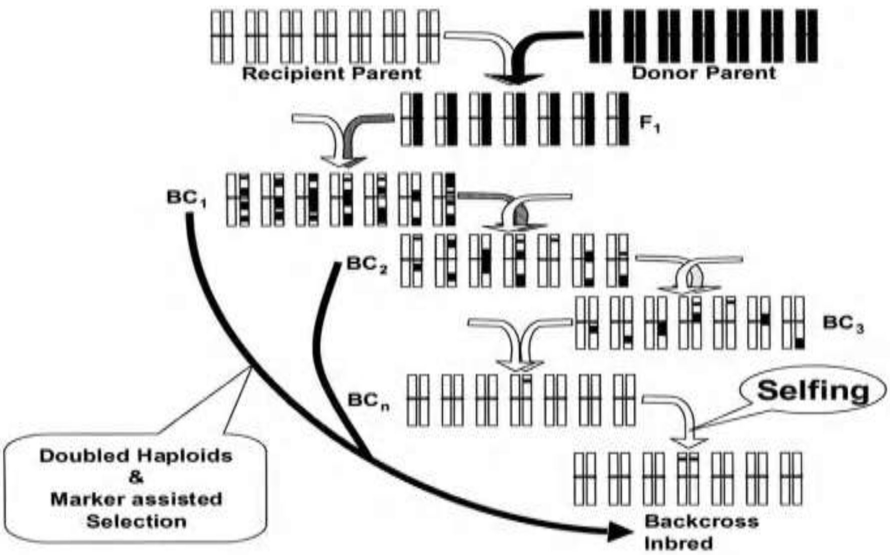

Figure 2. Development of doubled haploidy and Marker Assisted Selection in a rapid backcross conversion scheme (Thomas et al., 2003).

2.2. In studying the inheritance and combining desirable characteristics

When working with polyploidy plants, it is very useful to work at a lower ploidy level by haploid induction; studying the inheritance and combining desirable characteristics is much easier at a diploid level rather than in a tetraploid. This means that this technique is particularly efficient for autotetraploid plants such as potato.

2.3. In Distinguishing recessive mutants in the mutation breeding

Monohaploids have the advantage for the mutation breeder, because the recessive mutations (A-+a) are immediately discernable. This is not possible with a diploid which mutates from AA to Aa; self pollination of Aa gives rise to only one in four of the double recessive mutant. If a large population of haploid cells are available, then mutation induction and selection is possible on a large scale; so it is for example conceivable that resistance against phytotoxins could be selected for in a haploid cell population. An other advantage of mutation induction in haploid cells is that chimeras can not occur.

2.4. Obtaining super male plants in Asparagus

It is possible to obtain exclusively male plants of asparagus by haploid induction followed by chromosome doubling. Male plants of asparagus have a higher productivity and early yielding in the season than female plants. Female Asparagus plants are designated XX and males XY; by crossing XX and XY 50% female and 50% male plants are obtained. If haploids are produced from anthers of male asparagus plants these are either X or Y; chromosome doubling of Y results in super male plants with YY. They can subsequently be vegetatively propagated. If XX is crossed with YY then only males XY plants are obtained.

2.5. In the Obtaining Stable Somatic Hybrids

It is much better to work with haploid protoplasts rather than diploid ones for somatic hybridization. Fusion of two haploid protoplasts results in a diploid, while the fusion of two diploids results in a tetraploid.

2.6. In the Increasing Gen Transfer Efficiency

Efficiency of gen transfer by the gen transfer methods is higher in heterozygote tissues of corn than the homozygous tissues. Therefore, after obtaining genetically modified heterozygous plants of maize can be obtained genetically modified homozygous plants through the haploid techniques.

2.7. In the Gene Mapping

DH lines are ideal and the best populations for gene mapping due to their complete homozygosity, ability of repeating the experiment on several times and at different labs and less time required to produce a large DH population

3. OBTAINING HAPLOID PLANTS

Due to the mentioned using areas of the haploid plants, plant breeders have searched intensively for haploids that have occured spontaneously or attempted to make them artificially. The number of plant species known with haploids occoring spontaneously in vivo is more than 100, but as a rule the frequency is very low, 0.001 to 1 %. The most important sources of the spontenous haploids are apomixis and parthenogenesis.

Spontaneous occoring of haploid plants has been known since 1922, when Blakeslee first described this phenomenon in *Datura stramonium (*Blakeslee et al., 1922*)* ; this was subsequently followed by similar reports in tobacco (*Nicotiana tabacum*), wheat (*Triticum aestivum*) and several other species. However, spontaneous occurrence is a rare event and therefore of limited practical value. The potential of haploidy for plant breeding arose in 1964 with the achievement of haploid embryo formation from in vitro culture of Datura anthers (Guha and Maheshwari, 1964, 1966), which was followed by successful in vitro haploid production in tobacco. Many attempts have been made since then, resulting in published protocols for over 250 plant species belonging to almost all families of the plant kingdom (Kumar and Choudhary, 2020).

Under optimal conditions, doubled haploids (DH) have been routinely used in plant breeding for several decades, although their common use is still limited to selected species. There are several reasons for the limited uses of the doubled haploid. These might be categorized as biological, based on plant status (annual, biannual, perennial, authogamous, allogamous, vegetativelly propagated) and flower morphology or technical, which are the result of the feasibility and efficiency of DH induction protocol. Induction protocols substantially vary, in fact, not only among species but also among genotypes of the same species.

3.1.Obtaining Methods of Haploid Plants

Obtaining methods of haloid plants can be grupped as following;

In Vivo Occurrence of Haploid Plants

Spontaneous haploids

Hybridization

a) Intraspecific hybridization

b) Wide hybridization

Parthenogenesis

In Vitro Methods

Haploids from male gametes

a) Anther culture

b) Pollen/Microspore culture

Haploids from female gametes

3.1.1. In Vivo Occurrence of Haploid Plants

3.1.1.1. Spontaneous haploids

Naturally occurring haploids are in more than 100 species of angiosperms. e.g. wheatgrass, alfalfa, citrus, peach and trillium. These naturally occurring haploids have used in cultivar development. Chase (1952) was the first attemping to use haploidy in breeding. He selected the low frequency of parthenogenic haploids (egg cell develops into an embryo without fertilization) in maize and then applied chromosome doubling treatments to produce inbred lines. 'Kleine Liebling' is a haploid cultivar of Pelargonium and, 'Marglobe' is a DH cultivar of tomato (Dunwell, 2010).

3.1.1.2. Haploids through hybridization

3.1.1.2 (a). Intraspecific hybridization

Intraspecific hybridization of diploid material (*Haplopappus*), tetraploid material (*Pathenium, Sorghum, Sisymbrium* and alfalfa) and allopolyploid (*Aegilotriticum*) can results in haploids.

Haploids of sugar beet were identified by crossing diploid male sterile plants with green hypocotyls and tetraploid fodder beets homozygous for red hypocotyls.

In vivo DH induction in Corn

If maize plants are crossed with specific genotypes (inducer genotypes), a certain fraction of kernels has a haploid rather than a regular diploid embryo. Generally, kernels with a haploid embryo have a regular triploid endosperm. Therefore, such kernels have the same germination rate and vigor as those with a diploid embryo (Coe and Sarkar 1964).

There are two methods of in vivo haploid induction in maize leading to paternal (androgenetic) and maternal (gynogenetic) haploids, respectively. In the paternal haploids, the inducer is used as female and the donor plant as male parent. Thus the cytoplasm of paternal haploids originates from the inducer but the chromosomes exclusively from the donor plant. For production of maternal haploids, on the other hand, the inducer line is used as pollinator, leading to haploids with both cytoplasm and chromosomes from the donor. Both methods of in vivo haploid induction are much less dependent on the donor genotype than current in vitro techniques (Röber et al. 2005, Spitkó et al. 2006).

3.1.1.2 (a1). Induction of paternal haploids

The induction of gynogenetic haploids rely on properties of the mutant 'indeterminate gametophyte' caused by the recessive gene *ig* (Kermicle 1969). Multiple embryological abnormalities have been observed in homozygous *ig* plants. In some embryo sacs not all nuclei divide a third time, leading to various cytological irregularities including egg cells without a nucleus. After fusion of such an egg cell with one of the two paternal sperm cells, may develop a haploid embryo with the maternal cytoplasm and only paternal chromosomes. In selected inducer lines, the haploid induction rate ranges from 1 to 2% (Kermicle 1994; Schneerman et al. 2000). Paternal haploids have gained considerable importance for the creation of cytoplasmic male-sterile (CMS) female lines in commercial hybrid breeding. For this purpose, induction lines in various CMS-inducing cytoplasms have been developed (Pollacsek 1992; Schneerman et al. 2000). Using these CMS inducer lines, the transfer of the CMS cytoplasm into new breeding lines requires only a single induction cross rather than multiple backcross generations.

3.1.1.2 (a2). Induction of maternal haploids

Maternal haploid induction in maize (*Zea mays* L.) is a result of crossing within one species with selected inducing genotypes (line, single cross or population). It results in a majority of hybrid embryos and a smaller proportion of haploids with maternal embryos and normal triploid endosperms. The genetic strain Stock 6 with an haploid induction rate of up to 2.3% was first recognized inducer line (Coe, 1959). Today, modern haploid inducing lines display high induction rates of 8 to 10% (Geiger & Gordillo, 2009) (Table 2).

Table 2. Inducer lines used for the haploid induction in Corn

Inducer line	Haploid induction rate (&)	Reference
Stcks6	2	Coe,1959
WS14	2-5	Lashermes&Beckert,1988
KEMS	6	Shatskaya et al., 1994
RWS	8-23	Röber et al., 2005
PK6	6	Barret et al., 2008

They are routinely used in commercial DH-line breeding programs due to their high effectiveness and lower genotype dependence. In contrast to other induction techniques, no in vitro culture is needed, since kernels containing haploid embryos display a normal germination rate and lead to viable haploid seedlings. Haploid embryos can be selected early in the breeding process, based on morphological and physiological markers.

Procedures in the Obtaining maternal haploid maize plants

1) Inducing Maternal Haploidy

Induction of maternal haploidy is carried out in the field or greenhouse. Plants of the haploid inducer and of the source germplasm are grown under conditions allowing plant growth, pollination, and maturity of seeds. The planting dates of the inducer and the source germplasm should be adjusted so that the pollen set of the inducer is in synchronize with the silking of the source germplasm. Tassels should be removed from all source germplasm plants to avoid pollen contamination during pollination with the inducer. When inducing maternal haploidy, the following procedures are used (Prigge and Melchinger, 2012);

a. Before silk emergence, ears of source germplasm should be covered with ear bags to avoid uncontrolled pollination.

b. During anthesis, tassels of inducer plants should be covered with pollination bags in time to avoid contamination of their pollen with random pollen.

c. Plants of the source germplasm shall be pollinated with the pollen of the inducer line.

d. Pollinated ears are covered with pollination bags and the pollination bags are tightly fastened around the pollinated ear and the stalk, e.g. with a stapler.

e. Cross-pollinated ears of the source germplasm are harvested when they have reached physiological maturity.

For large-scale in vivo haploid induction, the inducer and source germplasm are grown in alternating rows in an isolated block (the number of rows of inducer and source germplasm depends on the pollen-shedding ability of the inducer) and all plants of the source germplasm are detasselled so that they are pollinated by wind with pollen from the inducer. This eliminates manual pollination.

2) Identification of Seeds with Haploid Embryo

In this step, seeds with a haploid embryo are visually separated from those with a diploid embryo using the R1-nj marker system carried by the inducers. The haploid seeds have an unpigmented (haploid) embryo and a purple (triploid) endosperm (Fig. 3), whereas normal F1 seeds have a purple (diploid) embryo and a purple (triploid) endosperm.) In addition, completely unpigmented seeds may occur at a very low frequency. Seeds with a haploid embryo are selected and the rest are discarded. Haploids have now been produced and the following two steps are only necessary if the aim is to produce double haploids.

3) Chromosome doubling in the maternal haploid plants

Haploid seedlings are weak and very sensitive to stress caused by colchicine treatment and transplantation. Therefore, they must be handled very carefully. To duplicate the chromosomes in the haploid plants, the following procedures should be followed

a. Haploid seeds are germinated in labeled germination trays with wet filter paper under controlled conditions in the dark with adequate moisture supply.

Fig 3. Seeds formed on the ear of the mother plants after pollination by inducer line. A: Haploid seeds with purple endosperm and normal embryo, B: Hybrid seeds with purple endospern and embryo, C: Seeds with normal endosperm and ambryo from crossing or selfing

b. When the coleoptiles of the seedlings are about 2 cm long, each seedling is individually removed from the tray, a few millimeters of the tip of its coleoptile is cut off with a scalpel or razor blade, and the seedling is placed in a mesh bag labeled with the appropriate identification number.

c. Place the mesh bags in the colchicine treatment container (for 1 L of 0.06% colchicine solution, dissolve 0.6 g of colchicine powder in 995 mL of deionized water and add 5 mL of DMSO to the solution). The solution is mixed on a thermolyne mixer for approximately 15 minutes).

d. Add colchicine solution to the treatment container until all seedlings are well covered. Personal protective equipment should be used for all steps involving colchicine.

e. Maintain seedlings submerged in colchicine solutions at room temperature (approximately 20°C) for 8 hours.

f. After treatment, the colchicine solution is drained from the treatment container and collected in specially designated waste containers. Treated seedlings are rinsed with tap water to remove residual colchicine. This is done by filling the treatment container with tap water until all seedlings are completely submerged, then draining the container and collecting the waste water.

g. Fill pots with sterilized soil and transfer treated seedlings to pots. Protective gloves should be worn when handling colchicine-treated seedlings.

h. The potted seedlings are placed in the greenhouse for approximately 10 days to allow them to recover from the colchicine treatment and grow to the three or four leaf stage. During this time, conditions favorable to seedling growth should be maintained.

4) Self-Pollination of Doubled Haploids for Production of DH Lines

In this step, the assumed doubled haploid plants are transplanted and self-pollinated to maintain and propagate seeds of the new DH lines. Typically, colchicine treatment does not result in uniform or complete doubling of chromosomes in all cells of a seedling. Some of the plants may have tassels that produce abundant pollen, while in most cases the tassels will have limited or no pollen-producing anthers. As a result, self-pollination can be extremely difficult.

The following procedures should be used when self-pollinating the suspected doubled haploid plants;

a. The seedlings are transplanted to the field or to larger pots where the plants can mature.

b. Standard crop management practices, including proper irrigation, effective weed and disease control, and appropriate fertilization, are used to provide favorable growing conditions for supposed DH plants.

c. Stem coloration of putative DH plants is monitored prior to flowering and plants with purple stem coloration are eliminated. These had been misclassified F1 plants.

d. Ear shoots (female flowers) will be covered before silk emergence.

e. Silk and anther emergence are monitored, tassels are covered with pollination bags in time for intended pollination and each plant is self-pollinated. Pollinated ears are covered with pollination bags for protection and stapled tightly.

f. Self-pollinated ears are harvested at physiological maturity.

3.1.1.2 (b). Wide Hybridization

Wide interspecific crossing has been shown to be a very effective method of haploid induction and has been used successfully in several crop species. It exploits haploidy from the female gametic line and involves both interspecific and intergeneric pollination. Fertilization of polar nuclei and production of functional endosperm can induce parthenogenetic development of haploid embryos that mature normally and are propagated by seed (e.g., potato).

In other cases, fertilization of ovules is followed by elimination of paternal chromosomes in hybrid embryos. The endosperms are absent or poorly developed, requiring embryo rescue and further in vitro culture of embryos (e.g. barley).

In barley, haploid production is the result of wide hybridization between cultivated barley (*Hordeum vulgare*, 2n=2x=14) as the female and wild *H. bulbosum* (2n=2x=14) as the male. After fertilization, a hybrid embryo is formed that contains chromosomes from both parents. During early embryogenesis, chromosomes of *H. bulbosum* are preferentially eliminated from the cells of the developing embryo, resulting in the formation of a haploid embryo and the failure of endosperm development. A haploid embryo is later extracted and grown in vitro (Figure 4). The 'bulbosum' method was the first haploid induction method to produce large numbers of haploids in most genotypes and was quickly incorporated into breeding programs(Kasha and Kao, 1970). Pollination with maize pollen could also be used for the production of haploid barley plants, but at lower frequencies.

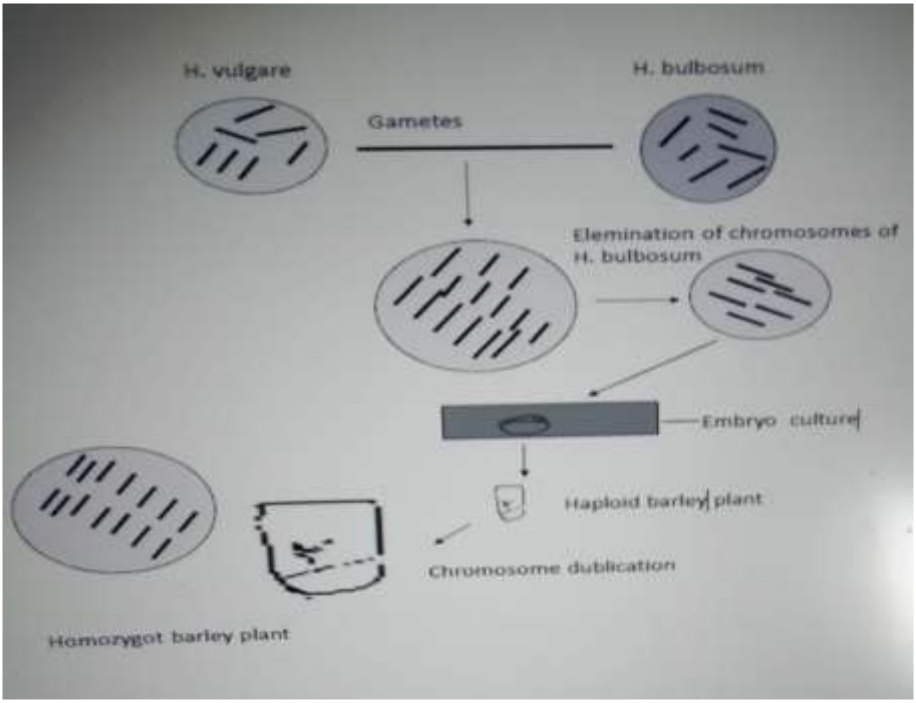

Fig 4. Aplication of Bulbosum technique

Paternal chromosome elimination has also been observed in interspecific crosses between wheat (*Triticum aestivum*) and *Hordeum bulbosum* (Barclay, 1975). This system was ineffective due to the presence of the Kr1 and Kr2 genes located on wheat 5A and 5B chromosomes, which significantly reduce the crossability between wheat and *H. bulbosum*.

After pollination, a hybrid embryo develops between wheat and maize, but in the further process the maize chromosomes are eliminated, so that haploid wheat embryos can be obtained. Such haploid wheat embryos usually cannot develop further if left on the plant because the endosperm does not develop in such seeds. By applying the growth regulator 2,4-dichlorophenoxyacetic acid in wheat plants, embryo growth is maintained to the stage suitable for embryo isolation and further in vitro culture. The maize chromosome elimination system in wheat allows the production of large numbers of haploids from any genotype (Laurie and Bennet, 1988; Comeau et al., 1988; Suenaga and Nakajama, 1989; Laurie and Reymondie, 1991).

Pollination with maize is also effective for inducing haploid embryos in several other cereals, such as barley, triticale (x Triticosecale), rye (Secale cereale) and oats (*Avena sativa*) (Wędzony et al., 2009). Similar processes of paternal chromosome elimination occur after the pollination of wheat with sorghum (*Sorghum bicolour* L. Moench) and pearl millet (*Pennisetum glaucum* L.R.Br.; Inagaki,2003). The following wide hybridizations are also giving the haploid embryos; *Triticum aestivum X Teosinte / Pennisetum glaucum / Pennisetum americanum L. /Sorghum bicolor / Imperata cylindrical /Coix lacryma-jobi/ Setaria italica/ Festuca arundinacea/ Cynodon dactylon/ Lolium temulentum/ Phalaris minor, Triticum durum X Zea mays /Imperata cylindrical, Avena sativa X Zea mays, Brassica napus X Orychophragmus violaceus, Brassica rapa X Isatis indigotica*.

Haploid production in cultivated potato (*Solanum tuberosum* L. ssp. tuberosum, 2n=4x) can be achieved by intergeneric pollination with haploid inducer clones of *S. phureja* (2n=2x). The tetraploid *S. tuberosum* produces an embryo sac containing one ovule and two endosperm nuclei, all with n=2x genetic constitution, while the diploid pollinator S. phurea produces two sperm with n=x or 2x genetic constitution. After pollination, dihaploid (2n=2x) embryos can develop from unfertilised ovules, supported by a 6x endosperm formed by the fusion of polar nuclei with the two reduced sperm cells. The frequency of dihaploid seeds is low; they must be selected from hybrid seeds containing 3x or 4x embryos developed from eggs (n=2x) fertilised by haploid (n=x) or diploid (n=2x) sperm (Maine, 2003). Dihaploid potatoes can be used for breeding purposes, including the introgression of foreign germplasm or selection at the diploid level, but such plants are not homozygous. Haploids play an important role in the potato breeding programmes of a number of companies, since they allow interspecific hybridisation that would otherwise not be possible due to differences in ploidy levels and endosperm balance numbers. The potato gene pool can be broadened and certain valuable traits, such as disease resistance traits from wild solanaceous species, can be more efficiently introduced into cultivated potatoes (Rokka, 2009).

There is a new method for producing haploid plants by manipulating a centromere-specific histone, CENH3. When the CENH3 mutant, which expresses abnormal CENH3, is crossed with the wild type, the chromosomes from the mutant are eliminated and then the haploids are finally produced (Fig. 5).This method could be applied to any plant because the CENH3 is universal in eukaryotes (Ishii et .al., 2016).

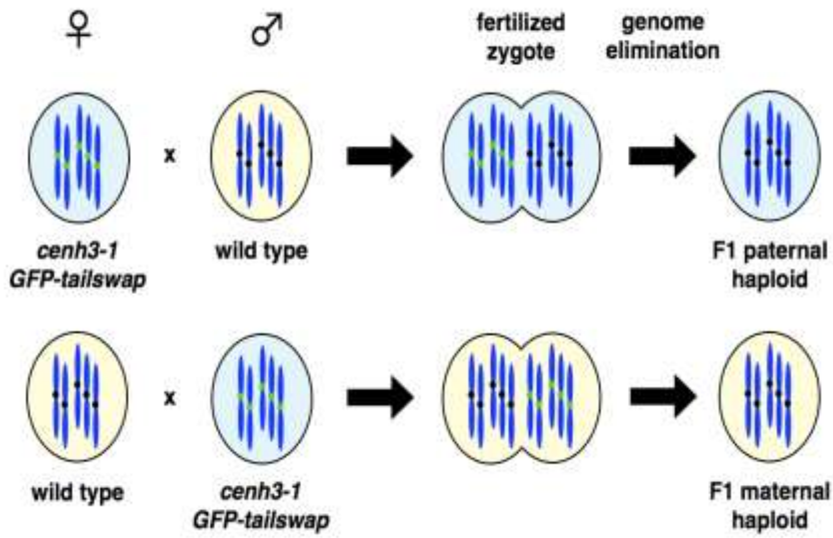

Figure 5. Haploid plant production through centromere-mediated genome elimination.

Currently, CENH3-mediated haploid induction protocols that result in a reasonable number of haploids have only been developed for the model plant A. thaliana (Karimi- Ashtiyani et al. 2015; Kuppu et al. 2015; Maheshwari et al. 2015; Ravi and Chan 2010). In crop species, an efficient CENH3-based haploid technology is not yet available, with the exception of maize, where a rate of up to 3.6% haploids has been achieved (Kelliher et al. 2016). Intensive research is currently focused on this goal, and two different approaches to CENH3-based uniparental genome elimination are being discussed. The first approach is based on the two-step strategy described by Ravi and Chan (2010), where lethal A. thaliana cenh3 knockout mutants were successfully rescued by transformation with modified CENH3 transgenes. Subsequently, the same group demonstrated that functional complementation of A. thaliana cenh3 null mutants and haploid induction was also possible with untagged natural CENH3 variants from progressively more distant relatives, such as different Brassica species (Maheshwari et al. 2015). This work also confirmed the previous suggestion that evolutionarily close CENH3s are capable of targeting centromeres in alien but not too distantly related species (Nagaki et al. 2010). The two-step strategy would most likely require the use of genetic modification and would result in transgenic haploidy inducer lines. Although the resulting haploid plants are expected to be non-transgenic due to the loss of chromosomes from the transgenic inducer plant, there is some concern, particularly in Europe, about the use of plant material that has undergone the process of genetic transformation in breeding programmes.

3.1.1.3. Parthenogenesis

Haploid plant production by parthenogenesis involves the culture of egg cells in the embryo sac without the involvement of sperm nuclei. Palmer and Keller (2005) distinguished parthenogenesis from gynogenesis, with the former involving "normal endosperm development and embryo formation in vivo", whereas in gynogenesis the endosperm degenerates over time and the embryo must be rescued under laboratory conditions. Chase (1949) produced doubled haploids in maize by parthenogenesis and used these haploids in his breeding programme. He used a colour genetic marker (dominant purple) in the pollinator to distinguish haploids (colourless) from diploids that had undergone chromosome doubling by injection of colchicine into the scutellar node of haploid maize plants. As parthenogenesis is rare in nature, it is difficult to distinguish between diploids and haploids. Therefore, genetic markers are usually used in pollinators for selection purposes, as described by Bordes et al. (1997) in maize.

Induction of parthenogenesis is usually achieved by delayed poliination, pollination with abortive pollen, exposure of plants to both higher and lower temperatures than the plant, use of irradiated pollen and gametocidal chemicals. Kihara (1940) increased the frequency of haploids in T. monococcum by delaying the time of pollination and showed that the two phenomena have a definite correlation. No haploids were produced in 41 individuals pollinated 3-5 days after emasculation, whereas there were three haploids amongst 8 individuals pollinated 9 days after emasculation.

The abortive pollen of the same species would be able to provide the hormonal stimulus for egg and endosperm development, although it may not be able to cause fertilisation (Kumar and Choudhary, 2020). This technique has been successfully used to produce haploids in a number of plants, such as rice (Nakamura 1933) and Nicotiana glutinosa (Webber 1933).

By exposing plants to both higher and lower temperatures than the plant normally experiences, the egg can be stimulated to develop parthenogenetically. Nordenskiold (1939) obtained a haploid plant of rye by higher temperature treatment, i.e. 41 ºC for 45 minutes for 21 hours after pollination. Povolochko (1937) obtained haploids of Nicotiana tabacum by subjecting the female parent to both high and low temperatures and pollinating with N. alata. Heat treatment of pollen has been successfully used to produce haploids in maize by Mathur et al. (1980).

The use of chemicals to treat pollen is also widely applied and has been used in maize (Deanon 1957) and Brassica (Kitani 1994). The genetic control of parthenogenesis has been identified in maize and barley, where the indeterminate gametophyte (ig) and hap initiator genes are able to induce parthenogenesis in maize (Kermicle 1969) and barley (Hagberg and Hagberg 1980), respectively.

Haploid plants can be obtained by pollination with irradiated pollen (Sestili and Ficcadent, 1996). In this application, irradiation destroys the generative function of pollen but does not affect its ability to stimulate egg cells, thus allowing the formation of parthenogenic embryos. Irradiation studies on pollen in vitro began at the end of the nineteenth century with the discovery of X-rays by Roentgen. The first studies were aimed at assessing the effects of radiation on pollen germination and pollen tube growth (Sestili and Ficcadent, 1996). However, the first evidence of haploid production was obtained in Triticum monococcum (Khiara and Katayama, 1932). This technique was later applied to several important commercial crops such as wheat (Snape et al., 1983) and barley (Powell et al., 1983). Turkoglu et al. (2007) tested different genotypes and doses of gamma radiation to produce haploid cotton plants using the pollination with the irradiated pollen. They obtained some doubled haploid cotton plants.

3.1.2. In vitro Methods

3.1.2.1 Haploids from male gametes

Androgenesis is the process of induction and regeneration of haploids and double haploids derived from male gametic cells (Hale et al., 2022).

Androgenesis is the most extensively studied and widely used technology for obtaining DHs (Wedzony et al., 2009). Most methods recommend two key in vitro steps: (1) induction of the androgenic process and (2) regeneration of H/DH plants. The induction phase is often preceded by pretreatment of plants, inflorescences/flowers or anthers. The regeneration phase is sometimes followed by an in vitro rooting phase. Finally, the resulting plantlets are acclimated to ex vitro conditions. In most cases, the chromosome constitution of haploid plants must be doubled by chemical treatment, but in some species "spontaneous duplication" of chromosomes occurs during culture at a rate high enough for practical purposes. The development of a microspore into a plant can occur directly, i.e. by the formation of an androgenic embryo, often referred to in the literature as an "embryo-like structure" (ELS). Under appropriate culture conditions, ELSs germinate directly into plants. Induced microspores can also form callus tissue that regenerates plants via somatic embryos or shoots. Often, all developmental pathways coexist in the same culture, and their proportions depend on the genotype and culture conditions used.

Due to its high efficacy and applicability in a wide range of plant species, it has outstanding potential for plant breeding and commercial exploitation of DH. It is well established for plant breeding, genetic studies and/or induced mutations of many plant species, including barley, wheat, maize, rice, triticale, rye, tobacco, rapeseed, other plants of Brassica and other genera. The method is based on the ability of microspores and immature pollen grains to change their developmental pathway from gametophytic (leading to a mature pollen grain) to sporophytic, resulting in cell division at the haploid level followed by the formation of calli or embryos. Androgenesis can be induced with in vitro culture of immature anthers or isolated microsopres from them.

3.1.2.1 (a) Anther culture

Anther culture is widely used techinque for doubled haploid production in crops. Good aseptic techniques are required, but the technique is generally simple and applicable to a wide range of crops. In general, haploid plants are regenerated in vitro from anther microspores and require chromosome doubling treatments (Figure 6). A few species, such as barley, regenerate large numbers of DHs as a result of induced chromosome doubling during early cell division of the microspores.

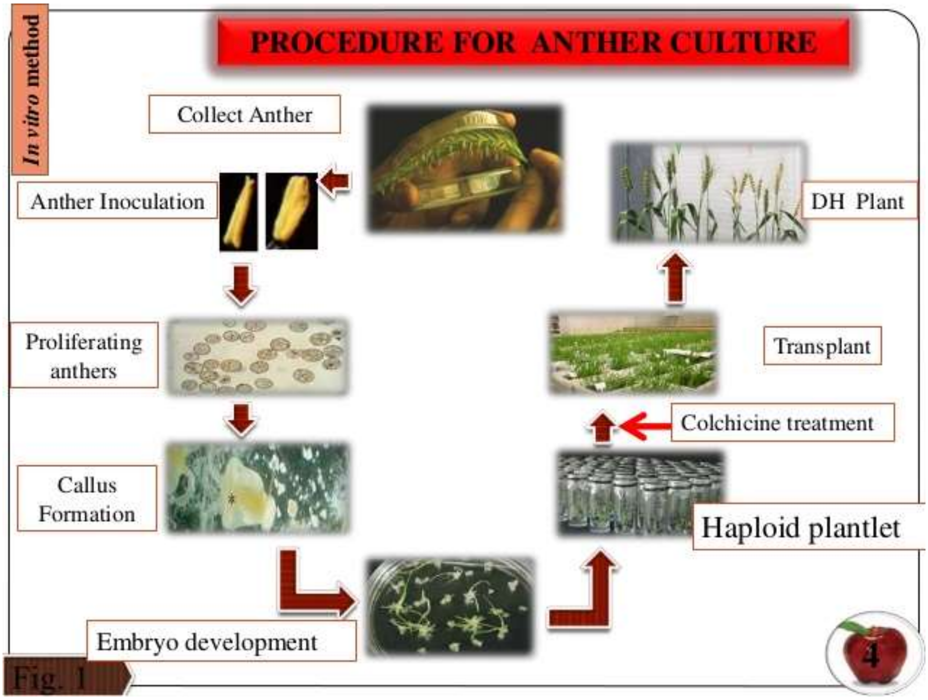

Figure 6. Procedure for Anther culture

Although the use of anther culture is widespread, the understanding of the processes involved has been poor. The presence of the sporophytic anther wall, which prevents direct access to the microspores contained within, has hampered studies. This has become an important issue. Although many species respond to anther culture, responsive genotypes can be a limiting factor and o develop genotype-independent methods, there is a need to study, understand, and manipulate microspore embryogenesis. In addition, to eliminate an intermediate callus phase that can promote gametoclonal variation among regenerants, there is a need to ensure direct embryogenesis. Bourgin and Nitsh (1967) first obtained haploid plants from the anthers of Nicotiana tabacum and N. sylvestris in 1967.

3.1.2.1 (a1) Factors Influencing Anther Culture

There are a number of factors that trigger androgenesis and with the improvement of various culture techniques, it has now become possible to induce pollen calli / embryos in a large number of plant species. These factors influencing pollen grains to enter a new developmental pathway can be genetic, physiological, physical, chemical, etc.(Maheshwari et al., 1980, 1982; Bajaj, 1983).

1) Genotype of donor plants:

Plant genotype plays an important role in determining the androgenetic response (Hatipoglu and Dogramacı, 1995; Hatipoglu et al., 1998; Başay and Ellialtıoğlu, 2013; Ahmed et al., 20-22; Lantos et al., 20-23). The large influence of the genotype effect on the response to in vitro cultivation is a major feature of anther cultivation. Embryo formation and the percentage of green plant regeneration in anther culture are strongly influenced by the genotype of the donor material. Hatipoglu et al. (1998) tested ten bread wheat genotypes for response to anther culture, and the rate of anther callus or embryo formation and the number of green plantlets per 100 anthers varied from 1.4% to 15.5% and from 0 to 7.5 green plantlets depending on the genotype (Table 3).

2) Effects of the donor plants's growing conditions

The physiological conditions of the donor plants, affecting number of pollen grains (Heberle-Bors 1985), endogenous hormone levels and nutritional status of anther tissues (Sunderland and Dunwell 1977), determine the success of the technique. The formation of embryogenic pollen grains in vivo and/or in vitro, characterized by a thinner exine structure, weak staining with acetocarmine, the presence of a vacuole and the absence of starch grains,

Table 3. Effects of genotype on the rate of anthers forming callus or embryoid and number of regenerated green plantlets per 100 anthers in anther culture of bread wheat

Genotype	Rate of anthers forming callus or embryoid (%)	Green plantlets/100 anther
BR124	1.4	0.0
Hahn	4.7	3.7
Tuıcm 74849	9.2	6.2
Bow’s	10.4	6.4
Atilla cm 85836	15.5	7.5
Buc	1.4	1.5
Furry’S’	4.4	3.7
Ka’S’/Nac	9.8	4.1
Hd2329	1.9	0.9
Bow/Na	3.4	1.6

seems to be related to a nitrogen starvation phenomenon (Heberle-Bors, 1989). Considerable seasonal variation in the response of anthers has been observed in numerous genotypes (Valdislavovna, 2015). Vasil (1980) observed that anthers from field-grown plants had a better response than anthers from greenhouse grown plants In addition, anthers taken from the first flush of flowers in the season have been found to give a better response (Sunderland 1971). Dunwell (1981) showed first that bothphotoperiod and light intensity affected the yield of micropore embryos and plantlets in tobacco. The influence of the temperature at which the donor plants are grown on the culture response has been demonstrated in studies of barley (Foroughi-Wehr and Mix 1976), oilseed rape (Keller and Stringham 1978; Dunwell and Cornish 1985), turnip (Keller et al. 1983), and wheat (Lazar et al. 1984), although optimal growth conditions appear to vary between species. The nitrogen nutrition of the plant also greatly affects the yield of microspore embryos (Tsay 1981), with "nitrogen-starved" plants giving better results than those supplied with fertilizer. Although the physiological state of the donor plant can dramatically affect the in vitro response of anthers, this parameter has only been studied in herbaceous plants because of the difficulty in determining it in woody plants.

3) The developmental stage of the microspores

The developmental stage of the microspores is a very important factor for the induction of callus or embryo from anther (Germana, 2011). It was found that the middle mononuclear stage is optimal for the culture of anthers (Figure 7).

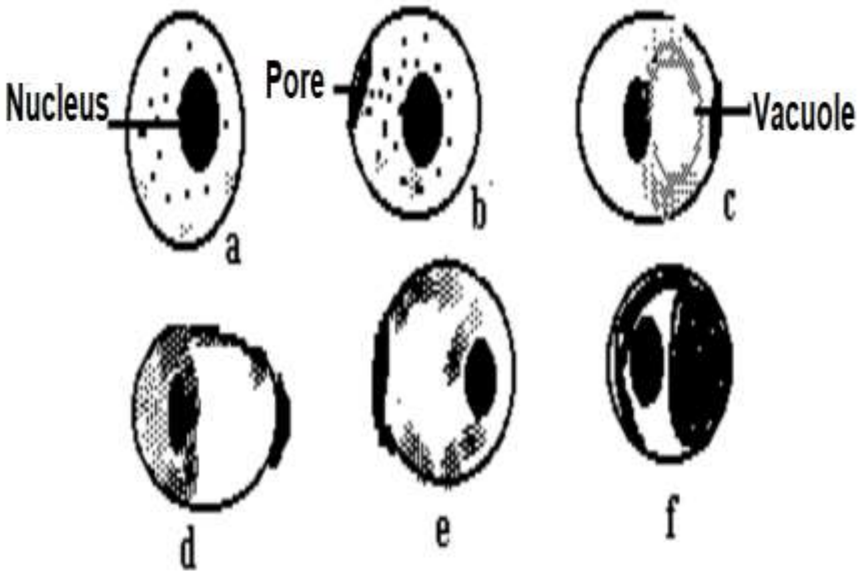

Figure 7. Development stages of microspore a. early uninucleate stage, b. Early-Mid uninucleate stage c. Mid-uninucleate stage, d. Late uninucleate stage, e. Starting first mitose f. binucleate stage (Hatipoğlu, 2008)

In hexaploid wheat, the frequency of green plantlets was higher in the middle and late uninucleate stage than in the early uninucleate stage, while the frequency of albinos was the same in both stages (Henry and De Buyser, 1990). The microspore stage in the anthers could be determined by microscopic examination of anther preparations. Acetacarmine staining is performed on fresh anthers from the central flowers of each spike. A correlation is made between the developmental stages of the microspores and various morphological characteristics such as: length of the bud, distance between the ligules of the flag leaf and the next lower leaf, color and position of the upper part of the anthers in relation to the glumes, and texture of the spike (Table 8)

Table 8. Morphological markers for microspore developmental stage in different crop species.

Plant Species	**Morphological marker for proper microsporec stage**	**Reference**
Wheat	Tip of the spike at the penultimate leaf	Henry and De Buyser (1990)
Barley	Distance between the ligules of the flag leaf and the next lower leaf should be 3-6 cm	Cistue et al. (2003)
Rice	The distance of the flag leaf auricle to that of the next leaf should be between 5 to 9 cm	Zapata-Arias (2003)
Maize	just before emergence of the tassel from the whorl	Mohammadi et al. (2007)
Timothy	heads extending 3-4 cm out of their sheaths	Pulli and Guo (2003)
Perennial ryegrass	Half to two third of the spike has emerged from the sheath	Andersen (2003
Rapeseed	Buds with 3.2-3.3 mm length	Custers (2003)
Potato	Buds with 4-6 mm length	Tai and Xiong (2003)
Tobacco	Buds with 10-11 mm length	Touraev and Heberle-Bors (2003)

5) Pretreatment

It has been observed in many genotypes that physical or chemical pre-culture treatments applied to excised flower buds, whole inflorescences, or excised anthers prior to culture act as triggers to induce the sporophytic pathway., thereby preventing the development of fertile pollen (gametophytic pathway) (Germana, 2011) . Pretreatments like chilling, high temperature, high humidity, water stress, anaerobic treatment, centrifugation, sucrose and nitrogen starvation, ethanol, c-irradiation, microtubule disruptors, electrostimulation, high medium pH, heavy metal treatment are popular approaches in anther and microspore culture. Temperature shock is considered to be the most effective treatment to induce pollen embryogenesis development. Depending on the genotype, the optimum temperature and duration of pretreatment vary. For example, Dunwell et al (1983) founda that among three cultivars (Tower, Willi and Duplo) of spring rape (*Brassica napus ssp. oleifera*), the highest yield (equivalent to 1.1 embryo per cultured anther) was obtained from anthers of the cv. Duplo after a 3-day treatment at 35 °C, while the yields from the other cultivars were much lower and relatively unaffected by the 35 °C treatment. Cold pretreatment (4 °C for 2–3 weeks) is employed routinely in the anther culture of many crops, and its effect is also genotype-dependent . Wheat anthers must be pretreated at 4 °C for 2-3 day (Xynias et. Al., 2001), barley at 4 °C for 28 days (Lazaridou, 2005) , maize at at 7 °C for 7-10 days (Hosseini et al., 2014; Barnabas, 2003). Nutrient starvation, especially for sugars and nitrogen, has been routinely used to induce pollen embryogenesis in some species (Kyo and Harada 1986). Although the mechanism of how stress affects pollen differentiation is not well understood, it appears to act by changing the polarity of the first haploid mitosis, involving reorganization of the cytoskeleton (Reynolds 1997), delaying and modifying pollen mitosis (two equal-sized vegetative-type nuclei instead of one vegetative and one generative), blocking starch production or dissolving microtubules (Nitsch 1977), or maintaining the viability of cultured P grains (Heberle-Bors 1985).

6) Surface sterilization, anther excision

Prior to anther removal, it is necessary to remove surface contaminants (bacteria and fungi) by sterilization. In general, after pretreatment, flower buds are surface sterilized by immersion in 70% (v/v) ethyl alcohol for a few minutes, followed by immersion in a sodium hypochlorite solution (about 1.5% active chlorine in water) containing a few drops of Tween 20 for 10-15 minutes and then by three 5-min washes with sterile distilled water. In the last step, anthers are excised aseptically from the flower and placed onto the medium. An exception are barley spikes, which are sterilized only by being sprayed with 70% ethanol (Cistue´ et al. 2003) or by immersion for 5 min in ethanol 70%, followed by rinsing in sterile distilled water (Jacquard et al. 2003). Injures to anthers during excision should be avoided in order to prevent somatic callus production from antherwall tissues. When the anther sizes are minute, such as in Brassica and Trifolium, their extraction can be performed under a stereoscopic microscope (Bhojwani and Razdan 1983).

7) Medium

The composition of the culture medium plays a key role in the induction of microspore embryogenesis. The different genotypes show very different basal medium requirements to induce pollen-derived plant formation. The nutritional requirements of excised anthers are much simpler than those of isolated microspores. The most commonly used basal media for anther culture are N6 medium (Chu, 1978), MS medium (Murashige and Skoog 1962), Nitsch and Nitsch medium (1969), and B5 medium (Gamborg et al. 1968), but there are many others. In general, half strength MS salt mixtures are suggested for Solanaceae and N6 medium for cereals.

For embryo production in anther culture is a carbohydrate source essential because of its osmotic and nutritional effects (Sen and Singh, 2011). The influence of carbohydrate concentration is probably related to the regulation of osmotic pressure during the induction phase, as high concentrations of the carbon source appear to be detrimental later in the culture period. Sucrose is the major translocated carbohydrate in plant tissues and is the most commonly used carbon source in anther culture, usually at levels of 2-4%. High levels of sucrose (6-17%) are required in those species (e.g. Gramineae, Cruciferae) in which mature pollen is shed in the tricellular state, whereas for those in which mature pollen is bicellular (e.g. Solanaceae) lower levels, such as 2-5%, are usually beneficial. Sucrose is heat labile and autoclaved media contain a mixture of sucrose, D-glucose and D-fructose. Maltose has been successfully used to replace sucrose in barley anther culture, usually at a concentration of 62 g/l in the induction medium and half that amount in the regeneration medium. Maltose has also been added to anther culture medium of other cereals suc as wheat, triticale, rye and rice at concentrations ranging from 60 to 90 g/l .

The effects of plant growth regulators have been extensively studied in anther culture. Although a few model species (e.g., most members of Solanaceae) do not require the addition of auxin to the induction medium and induction occurs on simple media, the presence of growth regulators is critical for microspore-derived embryo production in the majority of plant species, especially the recalcitrant ones.

The type and concentration of auxins determine the pathway of microspore development, with 2,4-dichlorophenoxyacetic acid (2,4-D) inducing callus formation and indole-3-acetic acid (IAA) and naphthaleneacetic acid (NAA) promoting direct embryogenesis. Gibberellins and abscissic acid were occasionally added to the media.

The addition of activated carbon (0.5-2 g/l) to the medium increases the efficiency of microspore embryogenesis in several species. An increase in the reaction ratio of tobacco anthers from 41 to 91% was obtained by supplementing the basal medium with 2% charcoal. It appears that the charcoal acted by removing inhibitory substances from the medium and, presumably, from the anther wall, and by regulating the levels of endogenous and exogenous growth regulators.

The addition of antioxidants and activated charcoal is often useful in some genotypes since it reduces tissue browning caused by phenols. The addition of other substances such as glutamine, casein, proline, biotin, inositol, coconut water, silver nitrate (ethylene antagonist) and polyvinylpyrolidone has been reported. In addition, the addition of exogenous aliphatic polyamines (PAs) to the culture medium has been found to increase the number of pollen-derived embryos in some crop species.

In the anther culture of many cereal species, it was found that effect of co-cultivation with ovary tissues was benefical due to stimulatory role of arabinogalactans (Broughton, 2008) . pH is another factor influncing the gametic embryogenic process. In anther culture, the pH of the media is in the acid range and usually adjusted to 5.7–5.8 before autoclaving.

Anther culture media are generally solidified by adding agar, but the beneficial effect of other solidifying agents, such as starch (potato, wheat, corn or barley starch), gelrite, agarose and ficoll, has been reported. Hatipoglu and Dogramacı (1995) faound that wheat starch can be used instead of agar in the anther culture of wheat (Table 9).

Table 9. Effect of solidifying agent on ratio of reacted anthers and plant regeneration

Solidifying agent	Ratio of anthers with callus or embryoid (%)	Green plantlets per 100 anther
Agar	6.5	7.0
Wheat starch	7.7	6.4
Maize starch	4.4	5.4

8) Culture conditions

Anther cultures are typically incubated at 24-27°C and exposed to light at an intensity of approximately 2,000 lux for 14 h per 24-h day, but culture conditions can vary depending on the species (Germana, 2011). However, optimal conditions need to be determined for each individual system (Bhojwani and Razdan 1983. Light is an environmental signal that regulates pollen morphogenesis in vitro. Regarding the effect of light quality on anther culture, the embryogenic induction of microspores is inhibited by high-intensity white light, whereas darkness or low-intensity white light is less inhibitory. Incubation of anthers in continuous darkness has occasionally been found to be essential. A period of alternating light and dark after the induction period has also been found to be beneficial in several species. The composition of the atmosphere in the culture vessel has not received much attention, although experiments on tobacco have shown its importance. The density of the culture (i.e., the number of anthers plated per volume of culture vessel or per unit volume of medium) and the manner in which the explant is placed on the medium have also been found to be critical in anther culture. Previous studies have examined the influence of anther orientation in tobacco (Misoo et al., 1981), rice (Yangn and Zhou, 1979), and barley (Shannon et al., 1985).

3.1.2.1 (a2) In Vitro Development of Microspores

Haploids originating from a microspore with a single nucleus can be produced in different ways (Pierik, 1987) (Figure 10):

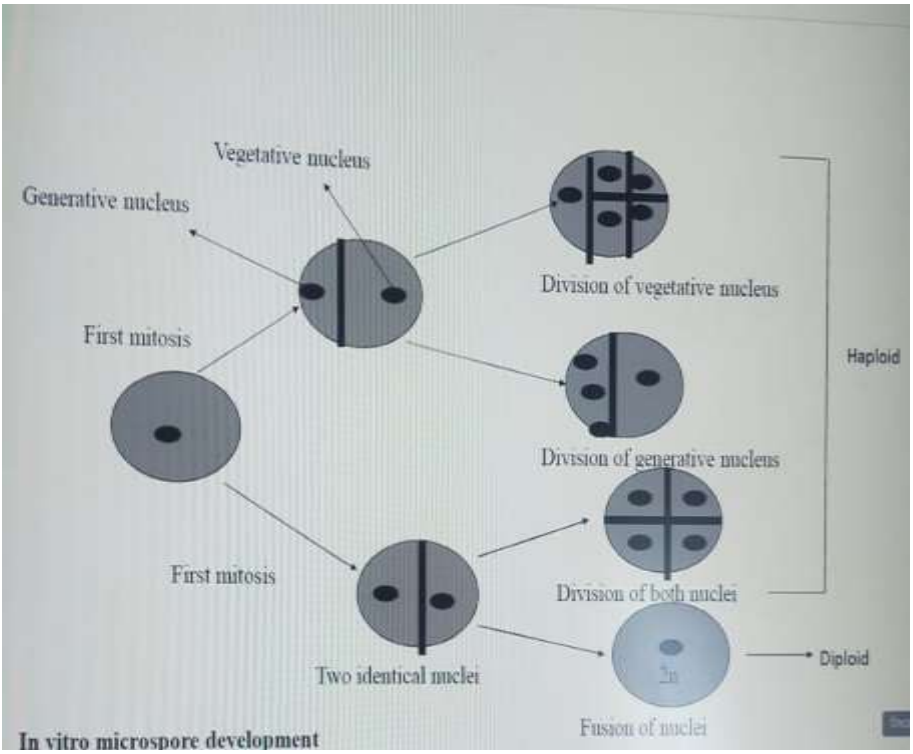

Figure 10. In vitro microspore devolopment

1. In many cases, after the first nuclear division (resulting in principle in a vegetative and a generative nucleus) of the microspore, the generative nucleus degenerates or becomes dormant and only the vegetative nucleus divides.

2. Sometimes, after the first division of the microspore, the vegetative nucleus degenerates or goes dormant and only the generative nucleus divides. This unusual occurrence is seen in Hyoscyamus.

3. Two identical nuclei are formed at the first division and both divide.This is called symmetrical microsporogenesis.

4. In practice, there is usually a mixture of all the above processes.

3.1.2.1 (a3) Regeneration of Haploid Plants From Microspores

Haploids can in principle be produced in 2 ways from isolated anthers (Hatipoglu, 2008)

1. Direct: An embryo differentiates directly from the microspore.

2. Indirect: First, a callus develops from the microspore, and then an embryo or adventitious shoot regenerates. This type of development is not favored because it usually produces a mixture of ploidy levels due to: the heterogeneous nature of the starting material (haploid and diploid), the addition of regulators, the spontaneous change from haploid to diploid. The haploids often cannot compete with the higher ploidy tissues.

3.1.2.1 (a4) Characterization of Regenerants: ploidy Analysis

Not only haploids have been obtained by in vitro anther culture.

The reasons for this last occurrence are ;

1.By endomitosis (from x to 2x and from 2x to 4x), instead of haploids, diploids and tetraploids are produced, which are homozygous.

2. By nuclear fusion of two identical nuclei within a single haploid microspore (x + x = 2x; 2x + x = 3x), diploids, triploids, etc... are produced, which are homozygous.

3. Regeneration from the septum and anther wall produces 2n individuals, which are heterozygous.

4. Heterogygous diploids develop by regeneration of non-reduced microspores.

5. A homozygous diploid is formed whenever there is spontaneous doubling of the haploid.

6. Abnormalities can occur during meiosis, resulting in different ploidy levels.

Chromosome numbers from root-tip cells of regenerated plantlets are counted using conventional cytological techniques. Ploidy level can be more easily assessed by flow cytometry analysis. Ploidy level can also be estimated by indirect methods, such as those based on chloroplast counts in stomatal guard cells and plastid dimensions.

3.1.2.1 (a4) Chromosome doubling in the case of haploidy

Spontaneous chromosome doubling has been observed during in vitro anther culture. The percentage of doubling is influenced by several factors, including the genotype, developmental stage of the microspores, type of pretreatment, and pathway of development. As examples of spontaneous doubling, average percentages of 70–90% have been reported in barley, 25–70% in bread wheat, 50–60% in rice, 50–90% in rye and 20% in maize (Maluszynski et al., 2003). Hatipoglu et al. (1998) found that spontaneous doubling ratio of green planlets from anther culture of different wheat hybrid genotypes varied from 16.7 % to 83.3% depending on the genotypes (Table 10).

Table 10 Ploidy levels of the regenerates from anthers of different hybrid wheat genotypes

Genotype	Ratio of regenerates with n=21 (%)	Ratio of regenerates with 2n=42 (%)
Seri-82x Kau’S’/Nac	66.7	33.3
Chil’S’ x Seri-82	44.4	55.6
Chil’S’ x TR 771778	75.0	25.0
84 ÇZT04 x Seri-82	83.3	16.7
Mexico-1 x Seri-82	16.7	83.3
Seri-82 x TR 771778	25.7	74.3
Seri-82 x Chil’S’	62.5	37.5

For those species with low doubling percentages, an efficient chromosome doubling protocol is required to convert sterile haploids regenerated into fertile, homozygous doubled haploid plants. Colchicine is the most widely used anti-microtubule agent in vivo and in vitro, but other doubling agents such as oryzalin and trifluralin have also been used. The success of the chosen protocol depends on toxicity and genome doubling efficiency of the doubling agents , which can vary depending on the genotype (80% barley, 60-70% bread wheat, 40-70% durum wheat, 50-80% triticale, 40% rice, 40% maize (Castillo et al., 2009; Maluszynski et al., 2003).

3.1.2.1 (b) Isolated microspore culture

Microspore culture is cultivation of the isolated microspores from the anthers prior to culture, whereas anther culture is the culture of the whole anther. Microspore culture has a number of special advantages over anther culture (Pierik, 1987);

1. The probabilty of regeneration of diploids is drastically reduced by elimination of diploid tissues (septum, anther wall, tapetum).

2. The anthesr can not acts as a barrier to the transport of nutrients from the medium to the pollen grains.

3. Inhibitors (ABA and toxic substances) are no longer a problem due to the elimination of the anther.

4. The formation of callus from the anther can be avoided, resulting in far fewer chimeras; if a callus develops from a single pollen grain, it will be of the same genotype, whereas if it develops from many pollen grains, chimeras can occur.

5. Direct transformation from pollen to embryo often occurs as a result of pollen isolation.

6. Pollen grains are more appropriate than anthers as starting material for mutation research and genetic manipulation.

7. Embryo formation is easier to observe in pollen grains than in anthers.

3.1.2.1 (b1) Protocol For Microspore Culture

1. **Growing donor plants:**

Healthy, pest-free donor plants are a prerequisite for a successful and consistent microspore culture response. Donor plants can be grown in the field, greenhouse, or in environmentally controlled growth chambers, planted with adequate spacing to allow for vigorous growth, watered regularly, and inspected and treated to minimize disease and insect infestations. The temperature at the growing area of the donor plants is plays a critical role in the response of the microspore culture. Cold temperature stress of the donor plants results in a higher frequency of microspore embryogenesis.

2.Harvesting floral organs:

The developmental stage of the microspores to be cultured is critical to success. Buds or tillers are typically harvested when the microspores are at the uninucleate to early binucleate stage. Developmental stage of the microspore can be determined by Acetocarmine staining .For many species, plant material is collected and used immediately, while for most cereals, tillers are selected, placed in nutrient solution, media, water, or inducer chemicals, and held for up to several weeks prior to microspore isolation. Most temperature pre-treatments are performed at 4-10°C, but short heat shock conditions of 33°C for 48-72 h can also be used.

3.surface sterilization:

Before the microspore isolation, tillers and buds are surface sterilized to eliminate bacterial or fungal contaminants. The most common surface sterilization protocols involve brief (1-2 min) immersion of the plant material in ethanol (70%), followed by immersion in sodium hypochlorite (6% or less) with a drop of tween for several minutes (up to 15 min), followed by several washes with sterile distilled water.

4) Isolating microspores:

Two main techniques are used to isolate microspores for culture. The first method involves mechanically crushing the surface-sterilized buds to release the microspores from the anthers, using a mortar and pestle or blender. The resulting slurry is then passed through series of filters to separate the anther wall and bud tissue from the microspores. The microspores are then collected by centrifugation. Maltose density gradients and sucrose gradients are used to separate the developmental stages of the microspores to obtain a more uniform and debris-free sample.

In the second method, shed microspore culture, anthers are extracted from buds, placed in a liquid medium, and the microspores are allowed to dehisce. The removal of somatic tissue from the microspore preparation is critical because its presence can adversely affect the microspores through the release of phenolic compounds and, in some cases, can produce diploid calli, embryos and plants, complicating the search for haploid or DH embryos and plants.

5.Culture and induction of microspores

Isolated microspores must be culture in a proper nutrient-rich medium and at appropriate culture conditions. The medium must include Macro- and micronutrients, vitamins, and a carbohydrate source. If contamination is a problem, antibiotics, such as cefotaxime, may also be added to the medium .Without stress, microspores follow their normal gametophytic pathway to form pollen grains. Stress can be applied by pretreatment of buds, tillers or isolated microspores and culture media. The stresses such as cold or heat shock, sugar starvation, and colchicine treatment are widely used, while γ-irradiation, ethanol stress, hypertonic shock, centrifugal treatment, reduced atmospheric pressure, feminizing agents, and abscisic acid were considered neglected stresses. Novel stresses included high medium pH, carrageenan oligosaccharides, heavy metal stress, inducer chemicals, and 2,4-D pretreatment. Pyramiding stresses may induce the switch to sporophytic development of microspores in recalcitrant species.

6.Regeneration of embryos

Microspores can follow a direct or an indirect route to develop into an embryo; the indirect route involves a series of irregular, asynchronous divisions that result in a callus, the callus undergoes organogenesis, and subsequently haploid embryos are formed. The direct and preferred route is similar to zygotic embryo development, where embryos develop directly and progress through the globular, cardiac, torpedo, and cotyledonary stages. Microspore-derived embryos are usually plated on a solid medium in the light for transformation of the embryos into plants.

3.1.2.2. In-Vitro Haploid And Doubled Haploid Plant Production Via Unfertilized Ovule Culture

The development of plants from unfertilized cells of the female gametophyte (embryo sac) through floret, ovary or ovule culture is an attractive alternative to anther culture for haploid production.

It was first reported in barley by San Noeum (1976). Since then, gynogenic haploids have been produced for 21 species belonging to 10 families (Wedzony et. Al., 2009). Gynogenetic induction using unpollinated flower parts has been successful in several species, such as onion, sugar beet, cucumber, squash, gerbera, sunflower, wheat, barley, etc., but its application in breeding is mainly limited to onion and sugar beet. Gynogenetic haploids mostly originate from unfertilized egg cells (parthenogenesis) as observed in barley, sunflower and sugar beet. However, in rice, gynogenetic haploids arise by synergid apogamy. The gynogenetic plants may arise by direct embryogenesis, or the gametic cells may form a callus followed by plant regeneration on another medium. The key factors affecting haploid plant regeneration through gynogenesis are summarised below:

1) Explant

Young flowers, ovaries or ovules have been used as explant to produce gynogenic haploids. In general, ovules attached to the placenta respond better than isolated ovules. In Helianthus annuus, ovule culture was superior to ovary or floret culture. In sugar beet, ovule culture was used most often, although in some cases ovary culture was better. In rice, the best results in gynogenesis were obtained when unhusked flowers with pistil and stamens attached to the receptacle were inoculated on liquid medium; the response was less effective when stamens were removed and was worst when single pistils were used. Similarly, in barley, whole florets (with or without stamens) inoculated vertically on solid medium performed better than randomly placed single pistils.

The stage of the female gametophyte in culture is crucial. In most cases, explants cultured at the almost mature embryo sac stage gave the best results (Figure 11). Rice is an exception where inoculation of ovaries at the 1-4 nucleate stage of female gametophyte development proved most responsive.

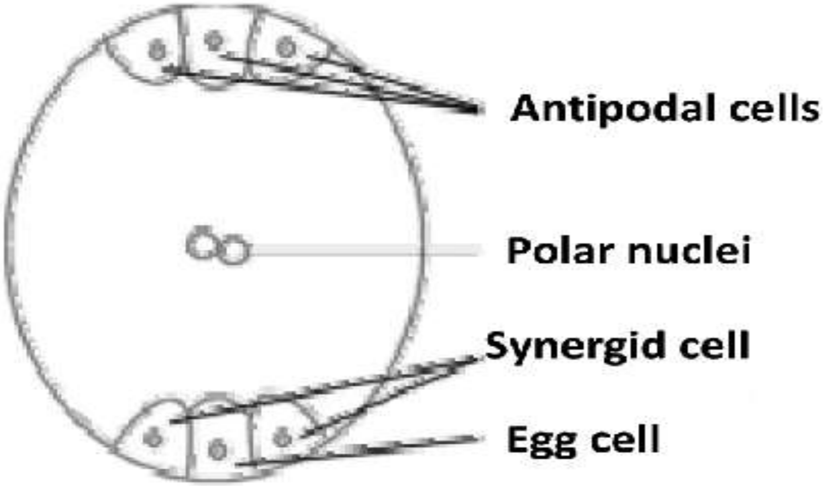

Figure 11. Embryo sac

2) Pre-treatment

A beneficial role of cold treatment on gynogenesis has been reported. Pretreatment of sunflower capitula at 4°C for 24-48 h before culture significantly increased the frequency of induction. It was observed a promoting effect of cold treatment of young panicles of rice, at 7°C for I day before ovary culture.

3) Culture medium

The culture media used to produce gynogenetic haploids vary widely. The most commonly used basal medium is N6 medium. However, N6 and MS were equally good for the production of maternal haploids of maize.

In general, sucrose is used at higher levels: 3-10% for barley, 8-14% for wheat, 5-12% for maize, and 3-6% for rice.

For graminaceous species, the induction medium is usually supplemented with 2,4-D (2 mg 1-1) or MCPA (0.125-0.5 mg 1-1).

The best combination of growth regulators for parthenogenetic haploid production in Beta vulgaris ovary/ovule culture was found to be 0.3 mg 1-1 BAP, 0.1 mg 1-1 NAA, and 0.05 mg 1-1 2,4-D.

In sunflower, however, a hormone-free medium gave a higher induction frequency than MCPA. Mostly solid media were used to induce gynogenesis. However, in rice, liquid medium was found to be superior to solid medium at the induction stage, whereas in maize, solid medium was more productive than liquid medium.

The cultures are initially kept in the dark. In vitro gynogenesis is a multi-stage process. The specific requirements of each stage should be carefully studied. For example, rice ovary culture involves at least two stages. The first stage of induction is characterized by float culture on liquid medium supplemented with exogenous auxin and cultured in the dark, while the second stage of regeneration requires transfer of callus to solid medium, reduction of auxin concentration, and keeping the cultures in the light.

REFERENCES

Ahmed Ahmed, H. A., Akdoğan G., Özcan S. F. and Barpete, S. 2022. Effect of Parental Genotypes and Their Reciprocal Crosses on Haploid Plant Production by Anther Culture and Confirmation of Double Haploids by Flow Cytometry in Bread Wheat. Journal of Agricultural Sciences (Tarim Bilimleri Dergisi), 28(3):363-371. DOI: 10.15832/ankutbd.825434.

Andersen, S.B. 2003. Doubled haploid induction in ryegrass and other grasses. In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), PP:179-183, Springer Science+Business Media New York.

Bajaj, Y.P.S., 1983. In vitro production of haploids. In: D.A. Evans, W.R. Sharp, P.V. Ammirato and Y. Yamada (Eds.), Handbook of Plant Cell Culture. Vol. I, pp. 228-287. Macmillan Publishing Co., New York.

Barclay, I.R. 1975. High frequencies of haploid production in wheat (Triticum aestivum) by chromosome elimination. Nature 256: 410-411.

Barnabas, B. 2003. Anther culture of maize (*Zea mays* L.). . In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), PP:,103-108, Springer Science+Business Media New York.

Barret, P., Brinkmann, M. and Beckert, M. 2008. A major locus expressed in the male gametophyte with incomplete penetrance is responsible for in situ gynogenesis in maize. Theor Appl Genet 117: 581 – 594

Başay, S. and Elliatıoğlu, Ş.Ş. 2013. "Effect of genotypical factors on the effectiveness of anther culture in eggplant (Solanum melongena L.)," *Turkish Journal of Biology*: Vol. 37: No. 4, Article 15. https://doi.org/10.3906/biy-1210-38

Bhojwani, S.S. and Razdan, M.K. 1983. Plant tissue culture: theory and practice, vol 5. Elsevier, New York.

Bhojwan, S.S. and Razdan, M.K. 1996. Plant Tissue Culture. Theory and Practie, a revised edition. Elsevier, Amsterdam.

Blakeslee, A. F., Belling, J., Farnham, M.E. and Bergner, A.D. 1922. A haploid mutant in the Jimson weed, Datura stramonium. Science 55: 646-647.

Bordes, J., de Vaulx, R.D., Lapierre, A. and Pollacsek, M. 1997. Haplodiploidization of maize (Zea mays L) through induced gynogenesis assisted by glossy markers and its use in breeding. Agronomie, 17, 291–297.

Bourgin, J. P. and Nitsch J. P. Obtention de Nicotiana hapldides A partir d'~tamines cultiv~esin vitro. (Production of haploid Nicotiana from excised stamens.) Ann. Phy.riol. Veg. 9:377-82.

Broughton, S. 2008. Ovary co-culture improves embryo and green plant production in anther culture of Australian spring wheat (Triticum aestivum L.). Plant Cell Tissue Organ 95:185–19

Castillo, A.M., Cistue, L., Valles, M.P. and Soriano, M. 2009. Chromosome doubling in monocots. In: Advances in haploid production in higher plants ,Touraev A, Forster B, Jain M (eds), pp: 329–338, Springer,

Chase, S.S. 1949. Monoploid frequencies in a commercial double cross hybrid maize, and its component single cross hybrids and inbred lines. Genetics, 34, 328–332.

Chu, C. 1978. The N6 medium and its applications to anther culture of cereal crops. In: Proc Symp Plant Tissue Culture. Science Press, Peking, pp 43–50.

Cistue, L. Valles, M.P., Echavarri, B.,Sanz, J.M. and Castillo, A. 2003. Barley anther culture. In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), PP: 29-34, Springer Science+Business Media New York.

Coe, E.H. 1959. A line of maize with high haploid frequency. Am Nat 93:381–382.

Coe, E.H and Sarkar, K.R. 1964. The detection of haploids in maize. J Hered 55:231–233.

Comeau, A., Plourde, A., Pieree, C. ve Nadeau, P.1988. Production of doubled haploid wheat lines by wheat x maize hybridization. Genome 30 Sublement 1: 482.

Custers, J.B.M. 2003. Micros pore culture in rapeseed (Brassica nap us L.). In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), PP:185-193, Springer Science+Business Media New York.

Deanon, J.R. 1957 Treatment of sweet corn silks with maleic hydrazide and colchicine as means of increasing the frequency of monoploids. Philipp Agric 41(7):364–377.

Dunwell, J.M. 1981. Stimulation of pollen embryo induction in tobacco by pretreatment of excised anthers in a water-saturated atmosphere. Plant Sci Lett 21:9–13.

Dunwell, J.M., Cornish, M. 1985. Influence of preculture variables on microspore embryo production in Brassica napus ssp. Oleifera cv. Duplo. Ann Bot 56:281–289.

Dunwell, J.M. 2010. Haploids in flowering plants: origins and exploitation, Review Article. Plant Biotechnology Journal 8:377–424

Foroughi-Wehr, B. and Mix, G. 1976. In vitro responses of *Hordeum vulgare* L. anthers cultured from plants grown under different environments. Environ Exp Bot 19:303–309.

Gamborg, O.L., Miller, R.A., Ojima, K. 1968. Nutrient requirement suspension cultures of soybean root cells. Exp Cell Res 50:151–158.

Geiger, H.H. and Gordillo, G.A. 2009. Doubled Haploids In Hybrid Maize Breeding. Maydica 54, 485–499.

Germana, M.A. 2009. Haploid and doubled haploids in fruit trees In: Advances in haploid production in higher plants, Touraev A, Forster B, Jain M (eds). Springer, Heidelberg, pp 241–263.

Germana, M.A. 2011. Anther culture for haploid and doubled haploid production. Plant Cell Tiss Organ Cult 104:283–300

Hagberg, A. and Hagberg, G. 1980. High-frequency of spontaneous haploids in the progeny of an induced mutation in barley. Hereditas 93(2):341–343.

Hale, B, Ferrie, A.M.R., Chellamma, S., Samuel, J.P. and Phillips, G.C. 2022. Androgenesis-Based Doubled Haploidy: Past, Present, and Future Perspectives. Front. Plant Sci. 12:751230. doi: 10.3389/fpls.2021.751230.

Hatipoglu, R. and Dogramacı, M. 1995. Die Wirkungen von Genotyp, Kulturmedium sowie Gelierstoff auf die Antherenkultur von Weizen (Triticum aestivum L.). Deutsch-Türkische Agrarforschung, Deutsch-türkisches Symposium, 12-17 September 1995, Ankara, S: 139-146, Herusgeber: Verband deutsch-türkischer Agrar- und Naturwissenschaftler e.V.,Verlag Ulrich E.Grauer-Stuttgart.

Hatipoğlu, R. 2008. Bitki Biyoteknolojisi.Ç.Ü. Ziraat Fakültesi Genel Yayın No: 190, Ders Kitapları Yayın No: 58. 7.baskı (İn Turkish) .

Hatipoğlu, R., Genç, I., Yağbasanlar, T., Ozkan, H., Celiktaş, N. and Toklu, F., 1998 . Production of Doubled Haploid Wheat Plants (*Triticum aestivum* L.) By Anther Culture. Agricoltura Mediterranean 28(3): 192-197.

Heberle-Bors, E. 1985. In vitro haploid formation from pollen: a critical review. Theor Appl Genet 71:361–374.

Henry, Y. and De Buyser, J. 1990. Wheat anther culture: Agronomic performance of doubled haploid lines and the release of a new variety ‘’ Florin’. In Biotechnology in Agriculture and Forestry V:13, wheat , Y.P.S. Bajaj (Ed.), pp: 285-352.

Hosseini, S.Z., İsmaili, A. And Mohammadi, P.P. 2003. Improvement of maize (Zea mays L.) anther culture embryogenesis and direct regeneration by differentplant growth regulators and gelling agent. Journal of Applied Biotechnology Reports Vol 1(1): 17-21.

Inagaki, M.. 2003. Doubled haploid production in wheat through wide hybridization. In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), P: 53-58, Springer Science+Business Media New York.

Iqbal, J. and Yousaf, U. 2018. Anther Culturing a Unique Methodology in Achieving Homozygosity. Asian Journal of Advances in Agricultural Research 8(2): 1-9.

Ishii, T., Karimi-Ashtiyani, R. and Houben, A. 2016. Haploidization via Chromosome Elimination:Means and Mechanisms. Annu. Rev. Plant Biol. 67:421–38.

Jacquard, C., Wojnarowiez, G. and Clement, C. 2003. Anther culture in barley. In: Doubled haploid production in crop plants, A manual, Maluszynsky M, Kasha KJ, Forster BP, Szaejko I (eds). PP: 21-27, Springer Science+Business Media, New York.

Karimi-Ashtiyani R, Ishii., T, Niessen, M., Stein, N., Heckmann, S., Gurushidze, M., Banaei-Moghaddam, A.M., Fuchs, J., Schubert, V., Koch, K., Weiss, O., Demidov, D., Schmidt, K., Kumlehn, J., Houben.

A. 2015 Point mutation impairs centromeric CENH3 loading and induces haploid plants. Proc Natl Acad Sci USA 112:11211–11216.

Kasha, K.J., Kao, K.N., 1970. High frequency haploid production in barley (Hordeum vulgare L.). Nature 225, 874–876

Kasha, K.J. and Maluszynski, M. 2003. Production of doubled haploids in crop plants. An introduction. In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), P: 1-4, Springer Science+Business Media, New York

Keller, W.A., Armstrong, K.C. and De La Roche, A.I.1983. The production and utilization of microspore-derived haploids in Brassica crops. In: Sen SK, Giles KL (eds) Plant cell culture in crop improvement. Plenum Press, New York, pp 169–183.

Keller, W.A., Stringham, G.R. 1978. Production and utilization of microspore derived plants. In: Frontiers of plant tissue culture, Thorpe TA (ed), Calgary University Press, Calgary, pp 113–122.

Kelliher, T., Starr, D., Wang, W., McCuiston, J., Zhong, H., Nuccio, M.L., Martin, B. 2016. Maternal haploids are preferentially induced by CENH3-tailswap transgenic complementation in maize. Front Plant Sci 7:414.

Kermicle, J.L. 1969. Androgenesis conditioned by a mutation in maize. Science 166:1422–1424.

Kermicle, J.L. (1994). Indeterminate gametophyte (ig): biology and use. In The Maize Handbook, M. Freeling and V. Walbot, eds, pp. 388–393, Springer-Verlag, New York.

Khiara, H. and Katayama, Y. 1932. Uber das Vorkommen von Haploider Pflanzen bei Triticum monococcum. Kwagaku 2: 408-410.

Kihara, H. 1940. Verwandtschaft der Aegilops-Arten im Lichte der Genomanalyse. Ein Überlick. Züichter 12: 49-62.

Kitani, Y. 1994. Induction of parthenogenetic haploid plants with brassinolide. Jpn J Genet 69(1):35–39.

Kumar, O. And Choudhary, M. 2020. Double Haploid: An Overview. Int.J.Curr.Microbiol.App.Sci 9(1): 1012-1029.

Kuppu, S., Tan, E.H., Nguyen, H., Rodgers, A., Comai, L., Chan, S.W., Britt, A.B. 2015. Point mutations in centromeric histone induce postzygotic incompatibility and uniparental inheritance. PLoS Genet 11:e1005494.

Kyo, M. and Harada, H. 1986. Control of the developmental pathway of tobacco pollen in vitro. Planta 168:427–432 .

Lantos, C..Jancsó, M.,Székely, Á., Szalóki, T.,Venkatanagappa, S., Pauk, J.2023. Development of In Vitro Anther Culture for Doubled Haploid Plant Production in Indica Rice (Oryza sativa L.) Genotypes. Plants 2023, 12, ://doi.org/10.3390/plants12091774.

Lashermes, P., Beckert, M. 1988. Genetic control of maternal haploidy in maize (Zea mays L.) and selection of haploid inducing lines. Theor Appl Genet 76:405–410

Laurie, D.A., Bennett, M.D., 1988. The production of haploid wheat plants from wheat × maize crosses. Theor. Appl. Genet. 76, 393–397.

Laurie, D.A. ve Reymondie, S. 1991. High frequencies of fertilization and haploid seedling production in crosses between commercial hexaploid wheat varieties and maize. Plant Breeding 106:182 -189.

Lazar, M.D., Schaeffer, G.W. and Baenziger, P.S. 1984. Cultivar and cultivar x environment effects on the development of callus and polyhaploid plants from anther cultures of wheat. Theor. Appl. Genet. 67:273–277.

Lazaridou, T.B., Lithourgidis, A.S., Kotzamanidis, S.T. and Ropak, D.G. 2005. Anther Culture Response of Barley Genotypes to Cold Pretreatments and Culture Media. Russ J Plant Physiol 52, 696–699.

Lichter, R. 1982. Induction of Haploid Plants From Isolated Pollen of Brassica napus. Zeitschrift für Pflanzenphysiologie 105 (5): 427-434.

Maheshwari, S.C., Tyagi, A.K.,Malhotra, K. and Sopory, S.K.1980. Induction of haploidy from pollen grains in angiosperms, the current status. Theor. App. Genet. 58: 193-206.

Maheshwari, S.C., Rashid, A. and Tyagi, A.K. 1982. Haploids from pollen grains – Retrospect and Prospect. Am. J. Bot. 69: 865-879.

Maheshwari, S.C., Tan, E.H., West, A., Franklin, F.C., Comai, L., Chan, S.W. 2015. Naturally occurring differences in CENH3 affect chromosome segregation in zygotic mitosis of hybrids. PLoS Genet 11:e1004970.

Maine, M.J. De.2003. Potato haploid technologies. In: Doubled Haploid Production in Crop Plants: A Manual, Maluszynski, M., Kasha, K.J., Forster, B.P. & Szarejko, I., pp. 241- 247, Kluwer Academic Publishers, ISBN 1-4020-1544-5, Dordrecht.

Maluszynski, M., Kasha, K.J., Szarejko, I. 2003. Published double haploid protocols in plant species. In: Haploid production in crop plants: a manual. Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds), Kluwer, Dordrecht, pp 309–335.

Mathur, D.S., Aman, M.A., Sarkar, K.R. 1980. Induction of maternal haploids in maize through heat- treatment of pollen. Curr Sci 49(19):744–746.

Misoo, S., Yokota, F., and Matsubayashi, M. 1981. Effects of inoculationways of anthers on the pollen mitosis and plantlet formation in tobacco anther culture. Rep Soc Crop Sci Breed Kinki 26:44–48.

Mohammadi, P.P., Moieni, A. And Jalali-Javaran, M. 2007. Colchicine induced embryogenesis and doubled haploid production in maize (Zea mays L.) anther culture. Iranıan Journal Of Bıotechnology, Vol. 5 (3): 140-146.

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plantarum 15:473–497.

Nagaki, K., Terada, K., Wakimoto, M., Kashihara, K., Murata, M. 2010. Centromere targeting of alien CENH3s in Arabidopsis and tobacco cells. Chromosome Res 18:203–211.

Nakamura, S. 1933. Haploid Plant in Oryza (Preliminary Note) (Japanese). Jap. Jour. Genetics. 8: 223-227.

Nature 225, 874–876.

Nitsch, C. 1977. Culture of isolate microspore. In: Reinert J, Bajaj YPS (eds) Applied and fundamental aspects of plant cell, tissue and organ culture. Springer, Berlin, pp 268–278.

Nitsch, J.P., Nitsch, C. 1969. Haploid plants from pollen grains. Science 163:85–87.

Nordenskiold, H. 1939. Studies of a haploid rye plant. Hereditas 25: 204-210.

Palmer, C.E. and Keller, W.A. 2005. Overview of haploidy. In: Haploids in Crop Improvement II, Vol. 56, C.E. Palmer, W.A. Keller, and K.J. Kasha, (eds), pp. 3–9. Heidelberg, Germany:Springer.

Pierik, R.L.M. 1987. In vitro culture of higher plants. Martinus Nijhoff Publishers, Boston.

Pollacsek, M. 1992. Management of the ig gene for haploid induction in maize. Agronomie. 12:247–251.

Povolochko, P. A. 1937.Experimental production of haploid plants in the genus Nicotiana. (In Russian; English summary). Bull, Appl. and Plant br., Ser. II, No. 7: 175-190.

Powell, W., P.D.S. Caligari and A.M. Hayter, 1983. The use of pollen irradiation in barley breeding. Theor. App!. Genet. 65: 73-76.

Prigge, V. and Melchinger, A.E. 2012. Production of Haploids and Doubled Haploids in Maize. In: *Plant Cell Culture Protocols*, Methods in Molecular Biology, vol. 877, V. M. Loyola-Vargas and N. Ochoa-Alejo (eds.), pp: 161-172. Springer Science+Business Media, LLC.

Pulli, A. and Guo, Y.D. 2003. Anther culture and isolated microspore culture in timothy. In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), PP:173-177, Springer Science+Business Media New York.

Ravi, M., Chan, S.W. 2010. Haploid plants produced by centromeremediated genome elimination. Nature 464:615–618.

Reynolds, T.L. 1997. Pollen embryogenesis. Plant Mol Biol 33:1–10.

Rokka, V.M. 2009. Potato Haploids and Breeding. In: Advances in Haploid Production in Higher Plants, Touraev, A., Forster, B.P., & Jain, S.M., pp. 199-208, Springer Science + Business Media B.V, ISBN 978-1-4020-8853-7.

Röber, F.K, Gordillo, G.A, Geiger, H.H. 2005. In vivo haploid induction in maize - Performance of new inducers and significance of doubled haploid lines in hybrid breeding. Maydica 50:275–283.

San Noeum, L.H., 1976. Haploids d Hordeum vulgare L. par culture in vitro non fecondes. Ann Amelior Plant 26: 751–754.

Schneerman, M.C., Charbonneau, M. and Weber, D.F. 2000. A survey of ig containing materials. Maize Genet Newsl 74:92–93.

Sen, C., Singh, P. 2011. Anther culture responses in boro rice hybrids. Asian Jour. Of biotech. 2011;3(5):470-477.

Sestili, S. and Ficcadent, N. 1996. Irradiated pollen for haploid production. In: In Vitro Haploid Production in Higher Plants, Vol. 1,263-274, S.M. Jain, S.K. Sopory & R.E. Veilleux (eds.), Sprlnger-Scıence+Busıness Medıa, B.V.

Shannon, P.R.M., Nicholson, A.E., Dunwell, J.M., Davies, D.R. 1985. Effect of anther orientation on microspore-callus production in barley (*Hordeum vulgare*). Plant Cell Tissue Organ 4:271–280.

Shatskaya, O.A., Zabirova, E.R., Shcherbak, V:S. and Chumak, M.V. 1994.Mass induction of maternal haploids in corn. Maize Genetics Cooperation Newsletter. 68:51

Snape, J.W., Parker, B.B., Simpson, E., Ainsworth, C.C., Payne, P.R. and Law, C.N.1983. The use of irradiated pollen for differential gene transfer in wheat (Triticum aestivum). Theor. App!. Genet. 65: 103-111.

Spitkó, T., Sági, L., Pintér, J., Marton, L.C. and Barnabás, B. 2006. Haploid regeneration aptitude of maize (*Zea mays* L.) – Lines of various origin and of their hybrids. Maydica 51:537–542.

Springer Science + Business Media B.V.

Suenaga, K. ve Nakajýma, K. 1989. Efficient production of haploid wheat (*Triticum aestivum*) through crosses between japanese wheat and maize (Zea mays). Plant Cell Rep. 8: 263 -266.

Sunderland, N. 1971. Anther culture: a progress report. Sci Prog (Oxford) 59:527–549.

Sunderland, N., Dunwell, J.M. 1977. Anther and pollen culture. In: Plant tissue and cell culture, Street H.E. (ed). Oxford, Blackwell,pp 223–265.

Tai, G.C.C. and Xiong, X.Y. 2003. Haploid production of potatoes by anther culture. In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), PP:,229-234. Springer Science+Business Media New York.

Thomas, W.T.B., Forster, B.P. and Gertsson., B. 2003. Doubled haploids in breeding. In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), P: 337-349, Springer Science+Business Media New York.

Touraev, A. and Heberle-Bors, E. 2003. Anther and microspore culture in tobacco. In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), PP:,223-228. Springer Science+Business Media New York.

Tsay, H.S. 1981. Effects of nitrogen supply to donor plants on pollen embryogenesis in cultured tobacco anthers. J Agric Res China 30:5–13.

Türkoğlu, Ş., Dolançay, A., Kaya, H., Toklu, P., Güler P.G, Hatipoğlu, R., Nasırcı, Z., Süllü, S. Ve Özbek, B.S. 2007. Pamukta (*Gossypium hirsutum* L.) Işınlanmış Polen İle Tozlama Tekniği Yoluyla Haploid Bitki Elde Etme Olanakları. Üzerinde Bir Araştırma. Türkiye VII. Tarla Bitkileri Kongresi, 25-27 Haziran 2007 Erzurum, C1: 356-359.

Vasil, I.K. 1980. Androgenic haploids. Int Rev Cytol Suppl 11A:195–223.

Vladislavovna, I.M. 2015. Effect of Growing Conditions of Rice Donor Plants on Anther Culture in Vitro. Journal of Agricultural Science and Technology A 5: 686-694.

Webber, J. M. 1933. Cytological features of Nicotiana glutinosa haploids. Jour. Agr. Res. 47: 845-867.

Wędzony, M., Forster, B.P., Zur, I., Golemiec, E. Szechyńska-Hebda, M., Dubas, E. and Gotębiowska, G. 2009. Progress in Doubled Haploid Technology in Higher Plants. In: Advances in Haploid Production in Higher Plants, A. Touraev, B.P: Forstyer and S.M. Jain (Edts.), pp: 1-33. Springer Science + Business Media B.V.

Xynias, I.N., Zamani, I.A., Gouli-Vavdinoudi, E. And Roupakias, D.G. 2001.Effect Of Cold Pretreatment And Incubatıon Temperature On Bread Wheat (*Triticum Aestivum* L.) Anther Culture. Cereal Research Communications Vol. 29 (3-4): 331-338.

Yangn, H.Y., Zhou, C. 1979. Experimental researches on the two pathways of pollen development in *Oryza sativa*. Acta Bot Sin 21:345–351.

Zapata-Arias, F.J. 2003. Laboratory protocol for anther culture technique in rice. In: Doubled Haploid Production in Crop Plants, A Manual, M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejko (Edts), PP:109-116, Springer Science+Business Media New York.

CHAPTER 3

THE SEED YIELD IN DIFFERENT SOWING DENSITY OF SOME LENTIL VARIETIES (LENS CULINARIS MEDIC.) IN KIRSEHIR ECOLOGICAL CONDITIONS

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1-INTRODUCTION

Rapidly increasing world population, decreasing production resources, lack of education, unconscious consumption, and imbalances in food distribution, adverse environmental conditions and regional wars are among the most important causes of humanity's hunger and nutrition problems. As a decisive way to solve nutrition problems, studies can be carried out to increase food sources, especially foods rich in protein, vitamins and minerals (Canbolat, 2014). Due to the limited arable agricultural areas, studies need to be carried out to increase crop production in parallel with the rapid increase in the world population, in order to obtain more and higher quality products from the unit area. Considering these problems that are growing day by day, the importance of edible legumes, which have high nutritional value, is better understood.

Our country, which is considered the natural gene center of coarse and medium-sized lentil varieties, is located on the Mediterranean and Near Eastern gene centers (Akdağ, 1996). Lentil, which is in the edible legume group in the world, has a very important place in human nutrition due to the high protein content it contains. Lentil; (Lens culinaris Medik) is a high energy and protein source food with 75% carbohydrate, 21% protein and 2% fat content and is rich in thiamine, iron, phosphorus and copper (Coşkuner and Karababa, 1998). Green lentils have an important place among edible legumes in Turkey, with a cultivation area of 16.3881 ha and a production of 20,000 tons (Anonim, 2016). Legume plants are able to fix nitrogen, which is free in the air, into the soil through bacteria called Rhizobium leguminosarum, with which they live in symbiosis on their roots. This ensures that the soil becomes rich in nitrogen for the plants that will be planted after legumes. They also decompose the N, Ca, P and K nutrients they contain in their roots in the soil and ensure that they remain in the root areas (Sepetoğlu, 2002).

Among other edible legumes, lentils take the first place in cultivation because they consume less water, fix nitrogen, and are resistant to low temperatures and drought. In addition to all these, lentil is a plant suitable for alternation with seeds and as a result, it is suitable for reducing fallow areas (Tantekin, 2008).

Genetic structure and environment are the two main factors that determine productivity. Applying the most appropriate cultivation techniques and growing varieties with high yield potential under changing ecological conditions is important to obtain quality and abundant products (Bozdemir, 2007). In order to obtain high yields of lentils in a region, it is essential to first select the appropriate varieties for the region, and predetermining the morphological and physiological characteristics of the varieties forms the basis for selecting superior yielding genotypes under certain environmental conditions. Considering the climate and soil requirements in lentil cultivation, it seems that our country has a great potential.

In addition to selecting varieties for each region, other agricultural practices must also be determined for those varieties. In addition to the selection of productive varieties for this region, seeding density is one of the most important agricultural practices. Sowing density is one of the important issues of cultivation technique. Therefore, determining the seeding density of different lentil varieties is an important issue. Kantar et al. (1994) reported that four different seed amounts (5, 6.5, 8.5 and 12.5 kg/da) were used in their research conducted under Erzurum conditions during the 1988-1990 production periods. The study was carried out on seed and total (seed-straw) yield of winter Red-51 lentil variety. They reported that seed and biological yield increased as the seed norm increased, but the rate of increase decreased after the 85 kg/ha sowing dose. Kose et al. (2017) two years to determine the effect of four different seeding densities (150-225-300-375 plants/m2) on the yield of five registered (Sultan-1, Meyveci 2001, Gümrah, Bozok and Karagül) and four local green lentil varieties in Yozgat conditions. In their study conducted over a period of time, the longest plant height (41.79 cm) and the highest number of pods per plant (43.42 pieces) were found in Local-3, the highest 1000 seed weight (65.7 g) in Karagül, and the highest seed yield (200.5 kg/da) in Bozok. Turk et al. (2003) reported that in their study conducted in Jordan between 1998 and 2001, they examined the effects of different seeding times and seeding densities (80, 100 and 120 plants/m^2) on yield and yield components in lentils. In his studies; They reported that seed weight, thousand-seed weight, number of primary branches, plant height and number of pods per plant decreased as plant density increased. They reported that seed yield is directly related to plant density and that as plant density increases, yield also increases. They reported that the highest yield was obtained from 120 plants/m², and that the increase in yield observed as the density increased was due to the presence of many plants with more pods per unit area. They reported that although the effect of the increase in productivity per unit area was due to the high number of pods per unit area, the increased plant density did not affect the number of pods. Karadavut et al. (2001) in a study conducted to examine the effects of three different row spacings (20, 30 and 40 cm) on yield and various yield components on Winter Pul-11, Winter Red-51 and local population lentil varieties; They reported that plant height and first pod height increased as the row spacing narrowed. They reported that biological productivity decreased as the distance between rows widened, and seed yield was not different between 20 and 30 cm row spacing in all varieties, while the highest yield was obtained from the Winter Pul-11 variety, whose thousand seed weight and harvest index were high in both years.

The aim of this study is to determine the effect of different seeding densities under Kırşehir ecological conditions on seed yield and some agricultural elements related to yield of nine lentil varieties commonly grown in the region.

2- MATERIAL VE METHOD

Material

This research was conducted in the production area of Ahi Evran University's Bağbaşı campus between 11 March and 29 June 2016, in Kırşehir ecological conditions. The research was carried out using nine green lentil varieties suitable for the region (Sultan-1, Fruiti 2001, Gümrah, Ankara Yeşili, Bozok, Karagül, Kayı-91, Ceren, Yusufhan) and 3 seeding densities (100-200-300 seeds/m²) has been carried out. It was examined at what seeding density the best values in terms of yield and yield elements of the varieties used were obtained.

Field trials were carried out in the trial area located on the Bağbaşı campus of Kırşehir Ahi Evran University during the 2016 summer green lentil growing period. The trial area is 5 km away from Kırşehir, its altitude is 1107 m, its latitude is 39º 9° north, and its longitude is 34º 10° east.

To determine the chemical and physical properties of the trial area soil, soil samples were taken from two different depths (0-30 cm and 30-60 cm) from different points of the trial area. The results are given in Table 1. When Table 1 is examined, it is seen that the soil is at a medium level in terms of organic matter, according to the results of the soil analysis. It is rich in potassium and phosphorus and moderate in calcium. The experimental area has a slightly alkaline and clay loam soil structure.

Table 1. Physical and chemical properties of field soil

Physical and chemical properties	Soil depth	
	0-30 cm	30-60 cm
pH	7.59	7.63
Salt %	0.02	0.02
EC (mmhos/cm)	0.52	0.56
Organic matter %	1.81	1.64
Phosphorus ((P2O5) kg da^{-1})	2.14	2.29
Potasium (K2O (kg da^{-1}))	66.62	51.47
Lime % (CaCO3)	27.9	28.39

The monthly total rainfall and monthly average temperature characteristics of the experimental site where the research was conducted are given in Table 2.

Table 2. Climate values of 2015-2016 year and the long-term average (LTA*)

	Temperature (c)			Rainfall (mm)		
Months	LTA	2015-2016	Difference	LTA	2015-2016	Difference
July	23.1	24.9	1.8	6.8	20.6	13.8
August	22.9	25.9	3	4.9	11.8	6.9
September	18.2	23.8	5.6	11.6	1	-10.6
October	12.3	14.6	2.3	27.8	30.8	3
November	6.2	8.4	2.2	36.4	8.8	-27.6
December	1.9	-1.2	-3.1	47.0	10.2	-36.8
January	-0.1	0.0	0.1	46.2	72.1	25.9
February	1.3	6.2	4.9	35.2	36.4	1.2
March	5.3	7.2	1.9	35.2	39.2	4
April	10.7	14.1	3.4	43.7	23.8	19.9
May	15.4	15.1	-0.3	44.3	95.8	51.5
June	19.6	21.3	1.7	36.8	16.1	-20.7
Total				375.9	366.6	
Average	11.4	13.3				

*LTA (lomg term Average, 1954-2016)

The experiment setup was completed in mid-March 2016. All observations were completed in July 2016. It is seen in Table 1 that the temperature averages with similar values compared to the long-term averages in 2016 are observed. The first precipitation was received in November.

In addition, it has been determined that the total amount of precipitation is 92.4 mm less than the precipitation statistics for LTA. In other words, the 2015-2016 seed production season can be evaluated as a dry season. It has been determined that relative humidity has a very low value in 2015-2016 compared to the LTA, depending on the rainfall received. (Demir, 2013)

Methods

This research was prepared according to the split-plot trial design in randomized blocks with 3 replications. The experiment was carried out using three seeding densities (100-200-300 seeds/m^2) and 9 green lentil varieties (Kayı-91, Sultan-1, Meyveci 2001, Gümrah, Ankara Yeşili, Bozok, Karagül, Ceren, Yusufhan). Seeding densities were placed in the main plots and varieties were placed in the sub-plots.

Each area was prepared as 0.80 x 5 m = 4 m2. Each plot consists of 4 rows and the distance between rows is kept as 20 cm. A distance of 40 cm was left between the plots and a distance of 3 meters between the blocks. Depending on the factor combinations, the experiment consisted of a total of 72 plots, 27 plots in each block.

The first soil preparation of the trial site was made in October 2015. In the experiment, seed sowing was done manually on March 11, 2016, in the rows opened with a marker machine.

According to the soil analysis results from the experiment, 15 kg/da of DAP (18-46-0) fertilizer was applied manually along with seeding. Weed control was done by hand plucking on 24.04.2016. Weed control was carried out for the second time, again by hand, on 08.05.2016.

The plants that were frequently checked in the trial area were harvested with a sickle on June 29, 2016. After leaving 0.5 m from the beginning of the plot and one row from the edges, the remaining plants were harvested by mowing with a sickle in the plots area (the two rows in the middle). The plots mowed with a sickle were placed in pre-prepared labeled bags and made ready for threshing. Then, the necessary measurements were made and blended in the laboratory.

Variance analysis of the data obtained was calculated using the MSTAT-C package program according to the 'split plots in random blocks' experimental design, and the averages were grouped with the "Duncan Multiple Comparison Test" (Steel and Torrie, 1960).

3-RESULTS AND DISCUSSION

Number of Plants per m²: In this study, the effect of three different seeding densities on the number of plants per m² of nine different green lentil varieties in Kırşehir ecological conditions was investigated. It was determined that the effects of green lentil varieties, plant density and seeding density x variety interactions on the number of plants per m² were statistically significant ($p<0.01$).

Table 3. Variance Analysis Summary of the Study

		F Value					
Source of Variation	Df	Number of Plants per M²	Number of Pods per Plant	Number of Seeds per Pod	Number of Seeds per plant	Seed weights per plant	Seed Yield
Replication	2	51.1[Ns]	0.6376 [Ns]	0.0239 [Ns]	1.0400 [Ns]	3.8139 [Ns]	5.3306[Ns]
Seeding Density (SD)	2	15713.6**	133.184**	2.5904 [öd]	177.5605**	143.189**	119.52**
Variety (V)	8	7.8222**	5.7410**	15.0284**	33.2664**	8.0096**	13.990**
SDXV	16	5.5136**	4.9923**	2.8603**	10.2059**	3.4899**	2.3001*
General	80						
Coefficient Variations(%):		11.1	9.98	9.99	9.61	15.45	8.83

* $P \leq 0.05$, P ** $p \leq 0.01$, Ns Non significant

The averages of seeding densities in this study are given in Table 4. In the study, three different seeding densities significantly affected the number of plants per square meter, and it was determined that the number of plants increased as the amount of seeds planted per square meter increased (Table 4.). Depending on the seeding density, the number of plants per square meter varied between 78.5 and 212.4 plants/m² (Table 4). The highest number of plants per square meter was determined as 212.4 plants/m² at 300 seeds/m², which was the highest seeding density applied in the study. The lowest sowing density applied per square meter (100 seeds/m²) was found to be 78.5 plants/m². As the seeding density increased, the number of plants harvested per unit area increased. Ağsakallı and Olgun (1999) reported that the number of plants per square meter was 206.4 in the Malazgirt-89 variety under Erzurum conditions. Turk et al. (2003) reported that in three different seeding densities (80, 100, 120 seeds/m²), they obtained the highest plant density from 120 plants/m² and observed that the yield increased as the plant density increased. In a study conducted by Tantekin (2008) under Diyarbakır ecological conditions, they reported that the number of plants per square meter of some lentil varieties at five seeding densities (150, 200, 250, 300 and 350 seeeds/m²) varied between 101.7-254.2 plants/m².

Table 4. The Number of Plants per M² in Green Lentil Varieties of Different Seeding Densities

Variety	Seeding Density (seed per m²)							
	100		200		300		Ort.	
Sultan-1	76.6	k	155.0	ıj	181.6	f-h	137.7	c
Bozok	81.6	k	140.0	j	188.3	e-g	136.6	c
Kayı-91	85.0	k	141.6	j	233.3	b	153.3	b
Meyveci 2001	65.0	k	161.6	h-j	213.3	b-d	146.6	bc
Gümrah	70.0	k	178.0	g-ı	225.0	bc	157.6	b
Ankara yeşili	78.3	k	185.0	e-h	196.6	d-g	153.3	b
Karagül	80.0	k	185.0	e-h	261.6	a	175.5	a
Yusufhan	85.0	k	185.0	e-h	205.0	c-f	158.3	b
Ceren	85.0	k	175.0	g-ı	206.6	c-e	155.5	b
Ort.	78.5	c	167.3	b	212.4	a		

*The difference between means marked with the same letter is insignificant (Duncan, $p \leq 0.05$)

When the varieties were examined in terms of the number of plants per square meter, it was observed that the difference between the varieties in terms of the number of plants per square meter was statistically significant (Table 3). It was determined that the number of plants per square meter varied between 136.6 plants/m² and 175.5 plants/m² depending on the seeding density among the varieties. While the highest number of plants per square meter was obtained from the Karagül variety with 175.5 plants/m², the lowest average value was obtained from the Bozok lentil variety (136.6 plants/m²). The variety that gave similar average values to the Bozok variety was the Sultan-1 lentil variety. These two varieties were found to have the lowest number of plants per square meter in the study. Varieties can produce different numbers of plants per square meter depending on the region. This reveals that the response of varieties to the negativities that occur during vegetation may be different (Demir, 2020a, Demir, 2020b Demir, 2020c).

The fact that the effects of seeding density in this study, the highest number of plants per square meter was determined in the Karagül variety with 261.6 plants/m² at a seeding density of 300 seeds/m². A similar result was detected from the Kayı-91 variety with 233.3 plants/m² at 300 seeds/m². It was observed that the Meyveci 2001 variety had the least number of plants per square meter with 100 seeds/m² and 65.0 (Table 4) (Yağmur and Kaydan, 2004).

When the number of plants per square meter was evaluated separately depending on the seeding density, it was observed that Kayı-91, Ceren and Yusufhan varieties were in the same group with the highest number of plants per 100 seeds/m², 85.0. The lowest number of plants was Meyveci 2001 variety with 65.0 plants (Table 4)

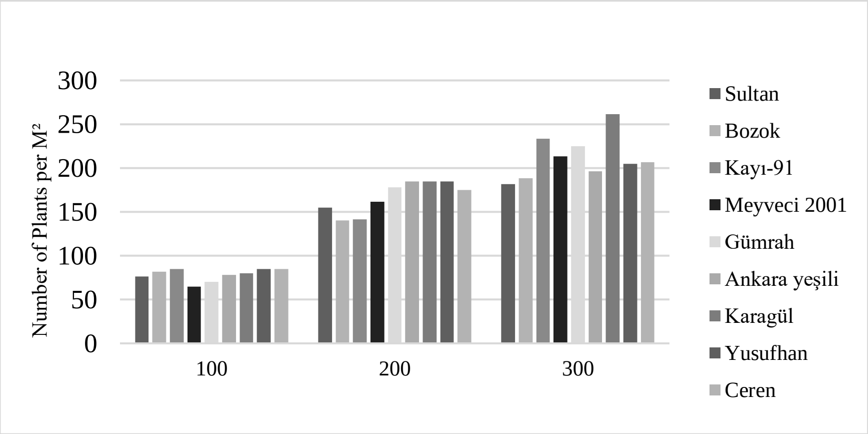

Figure 1. Effect of Seeding Density X Variety Interaction on the Number of Plants per M²

It was determined that the Yusufhan, Karagül and Ankara green varieties were in the same group with the highest number of plants at 200 seeds/m², with 185.0 plants. Bozok variety was determined with the least number of plants, with 140.0 plants. At 300 seeds/m², the highest number of plants was determined to be Karagül variety with 261.6 plants, and the lowest number of plants was determined to be Sultan-1 variety with 181.6 plants (Figure. 1, Table 4.).

Number of Pods per Plant

In this study, in which the effect of three different seeding densities on the number of pods per plant of nine different green lentil varieties under Kırşehir ecological conditions was investigated, it was determined that the interaction of seeding density, variety and seeding density x variety was statistically significant ($p < 0.01$) (Table 3).

When the research results are examined based on seeding density, the averages of the number of pods per plant are given in Table 5. The number of pods on the plant varied between 21.93 and 39.40, and the difference between the densities was found to be statistically significant. When the research was evaluated in terms of seeding density, it was seen that as the seeding density increased, the number of pods per plant decreased. While the highest number of pods per plant was obtained with 39.40 pods at a seeding density of 100 seeds/m², the lowest number of pods per plant was determined with 21.93 pods at a seeding density of 300 seeds/m². At a seeding density of 200 seeds/m², the average number of pods was determined as 27.81. The increase in seeding density caused the number of pods on the plant to decrease. This can be interpreted as a decrease in branching and a decrease in the number of pods per plant due to competition between the numbers of plants per unit area. As a matter of fact, many previous studies have revealed findings in this direction. Zulkadir et al. (2015) and Köse et al. (2017) reported that the number of pods on the plant decreased due to the increase in seeding density.

Similar findings were reported by Ağsakallı and Olgun (1999). Additionally, studies have reported that the number of pods per plant is significantly related to genotype and plant density. Tanyolaç (1992) and Mckenzie et al. (1986) report that as the number of plants per square meter decreases, the number of pods per plant increases; They argue that as the row spacing decreases, plants compete to benefit more from light and air, reduce branching and try to grow taller during competition, and therefore, it is an expected situation that the number of pods will be low in plants with low branching and skinnyness. In fact, another researcher, Idris (2008), stated that increasing the plant habitat, in other words, reducing the seeding density, increases the number of pods per plant and ultimately gives the highest seed yield (Yağmur and Kaydan, 2005)

Table 5 Number of Pods per Plant in Green Lentil Varieties of Different Seeding Densities

Variety	Seeding Density (seed per m^2)							
	100		200		300		Ort.	
Sultan-1	35.56	b-d	27.46	e-g	19.23	ı	27.42	d
Bozok	35.40	cd	29.40	ef	27.96	e-g	30.92	a-c
Kayı-91	31.56	de	27.46	e-g	20.43	hı	26.48	d
Meyveci 2001	38.06	bc	30.13	d-f	24.70	f-ı	30.96	a-c
Gümrah	41.20	b	25.26	f-h	22.43	g-ı	29.63	b-d
Ankara yeşili	35.73	b-d	27.20	e-g	20.46	hı	27.80	cd
Karagül	40.16	bc	25.40	f-h	20.53	hı	28.70	b-d
Yusufhan	47.26	a	28.30	e-g	19.20	ı	31.58	ab
Ceren	49.66	a	29.73	ef	22.46	g-ı	33.95	a
Averages	39.40	a	27.81	b	21.93	c		

*The difference between means marked with the same letter is insignificant (Duncan, $p \leq 0.05$)

A statistically significant difference was detected between the varieties in terms of the number of pods per plant. When the number of pods per plant was evaluated on a variety basis, it was determined that the averages varied between 26.48 and 33.95. While it was observed that the Ceren lentil variety had the highest average number of pods per plant with 33.95 pods, the lowest average number of pods per plant was found to be in the Kayı-91 variety with 26.48 units (Table 5). Erskine et al. (1989) reported that the number of pods in the plant is an important factor in determining the difference between lentil varieties. Stoilova (1999) evaluated the morphological, phenological and agronomic characteristics of 120 lentil materials in a two-year study in Bulgaria. They found significant genetic differences between genotypes in terms of the examined characters. In the study, they reported that the number of pods on the plant varied between 9.8 and 65.7. In their research, Biçer and Şakar (2003) found significant statistical differences among lentil varieties in terms of the number of pods per plant. Likewise, Aydogan et al. (2003), in a study they conducted on green lentils, found that the population showed a large variation in terms of the number of pods per plant.

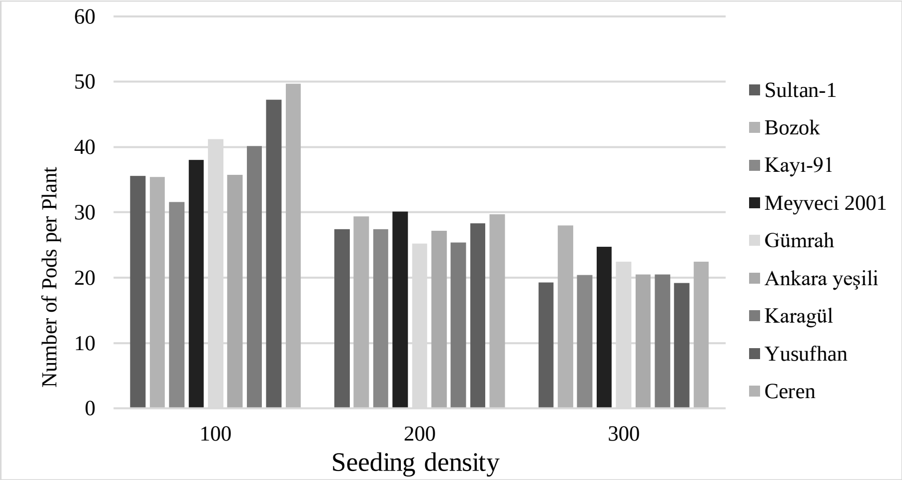

Figure 2. Effect of Seeding Density X Variety Interaction on the Number of Pods per Plant

When the number of pods per plant was examined in terms of the variety Ceren variety is followed by Yusufhan variety with 47.26 units per 100 seeds/m². It was found that the variety with the lowest number of pods on the plant was Yusufhan variety with 19.20 pods per 300 seeds/m² (Demir, 2021).

Number of Seeds in Pod: In this study, in which the effect of three different seeding densities on the number of seeds in broad beans of nine different green lentil varieties was investigated, it was found that the effect of seeding densities on the number of seeds in broad beans was not statistically significant. In addition, it was determined that the interaction of seeding density

In this study, when the effect of sowing density on the number of seeds in broad beans was examined, it was found that the effects of three different seed densities on the number of seeds in broad beans were not statistically significant. The averages of the number of seeds in broad beans are given in Table 4.20. It was found that the average number of seeds per pod in the three seeding densities used in the study varied between 1.05 and 1.08. It was determined that seeding density had no effect on the number of seeds in broad beans, and the average values were found to be close to each other.

A statistically significant difference was determined in terms of the number of seeds in broad beans among the varieties. When the number of beans in broad beans was evaluated on a variety basis, it was determined that the average number varied between 0.85 and 1.29. While it was found that Bozok lentil variety had the lowest average number of seeds per pod with 0.85, it was determined that Ceren lentil variety had the highest average number of seeds per pod with 1.29 (Table 4.20). The seed numbers of the other lentil varieties that were subject to the experiment were among the averages of these two types of broad beans.

Table 6. The Number of Seeds in Pod in Green Lentil Varieties of Different Seeding Densities

Variety	Seeding Density (seed per m^2)							
	100		200		300		Ort.	
Sultan-1	0.87	h-j	0.98	e-j	1.06	c-h	0.97	cd
Bozok	0.85	ıj	0.79	j	0.91	g-j	0.85	e
Kayı-91	1.30	ab	0.84	ıj	1.11	b-g	1.08	bc
Meyveci 2001	0.96	f-j	0.93	g-j	0.94	g-j	0.94	de
Gümrah	1.18	b-e	1.19	b-d	1.15	b-f	1.17	ab
Ankara yeşili	1.09	b-g	1.22	a-c	1.18	b-e	1.16	b
Karagül	1.00	d-j	1.02	c-ı	0.94	g-j	0.99	cd
Yusufhan	1.18	b-e	1.07	c-h	1.21	a-c	1.15	b
Ceren	1.23	a-c	1.41	a	1.22	a-c	1.29	a
Averages	1.07		1.05		1.08			

*The difference between means marked with the same letter is insignificant (Duncan, $p \leq 0.05$)

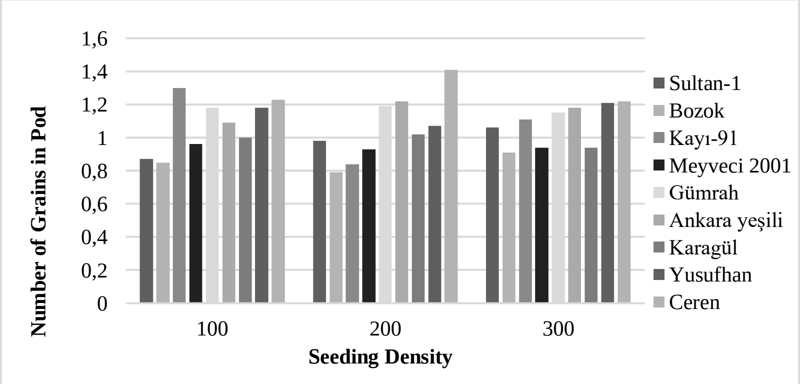

Figure 3. Effect of Seeding Density X Variety Interaction on the Number of seeds in pod

When the research results are examined in terms of the number of seeds in broad beans, the interaction between seeding density It has been determined that there is a Bozok lentil variety.

Number of Seeds per Plant: The effect of three different seeding densities on the number of seeds in the plant of nine different green lentil varieties has been researched in summer tie in Kırşehir ecological conditions. In the study, it was observed that the effect of sowing density, variety and sowing density x variety interaction on the number of seeds in the plant was statistically significant ($p<0.01$).

It was found that the average number of seeds per plant for the three seeding densities used in the study varied between 23.57 and 42.48. It has been determined that the effect of seeding density on the number of seeds in the plant is important, and the averages are found to have values that are far from each other. The highest average number of seeds per plant was 42.48, obtained from a seeding density of 100 seeds/m2. The seeding density with the lowest average number of seeds per plant was 300 seeds/m2. It can be seen in Table 4.22 that the application where the sowing density is 200 seeds per square meter has an average number of seeds in 29.16 plants.

When the number of seeds in the plant was evaluated on the basis of varieties, it was determined that the average number of seeds in the plant varied between 24.76 and 43.95. While it was found that the Kayı-91 lentil variety had the lowest average number of seeds per plant with 24.76 units, the highest average number of seeds per plant was found in the Ceren lentil variety with 43.95 units (Table 4.22). Among the other lentil varieties tested, Yusufhan variety followed the Ceren lentil variety in terms of the highest average number of seeds per plant, with 36.63 units. Other varieties were found to follow these two varieties in terms of average number of seeds per plant.

According to some researchers, lentil is a plant with low tolerance to high temperatures, high temperature and water stress increase the rate of flower drying and empty pods and reduce the plant's capacity to fill seeds in pods. This is especially important during flowering and pod setting periods (Biçer and Şakar, 2011). Turk et al. (2004) also mentioned; It has been reported that high temperature and low humidity at the time of pod filling in semi-arid conditions reduce the yield, and small, skinny and weak seeds are seen in lines with high pod number and seed number. Stoilova (1999) evaluated the morphological, phenological and agronomic characteristics of 120 lentil materials in a two-year study in Bulgaria. They found significant genetic differences between genotypes in terms of the examined characters. In the study, they reported that the number of seeds per plant varied between 9.7 and 75.5.

When the research results are examined, the interaction relationship between seeding density It has been determined that there is a Karagul lentil variety. This shows that each variety responds differently to different seeding densities (Yagmur et al., 2017)

Table 7. Number of Seeds in the Plant in Green Lentil Varieties of Different Seeding Densities

Variety	Seeding Density (seed per m^2)							
	100		200		300		Ort.	
Sultan-1	33.53	ef	26.53	g-j	20.60	jk	26.88	ef
Bozok	36.83	de	27.06	g-ı	25.56	g-k	29.82	de
Kayı-91	27.56	g-ı	23.96	h-k	22.76	I-k	24.76	f
Meyveci 2001	36.76	de	28.76	f-ı	23.56	h-k	29.70	de
Gümrah	48.46	c	29.20	f-h	24.86	h-k	34.17	bc
Ankara yeşili	40.83	d	31.00	Fg	24.10	h-k	31.97	cd
Karagül	37.23	de	26.36	g-j	19.60	k	27.73	ef
Yusufhan	57.26	b	29.13	f-h	23.50	hk	36.63	b
Ceren	63.83	a	40.46	d	27.56	gı	43.95	a
Averages	42.48	a	29.16	b	23.57	c		

*The difference between means marked with the same letter is insignificant (Duncan, p≤0.05)

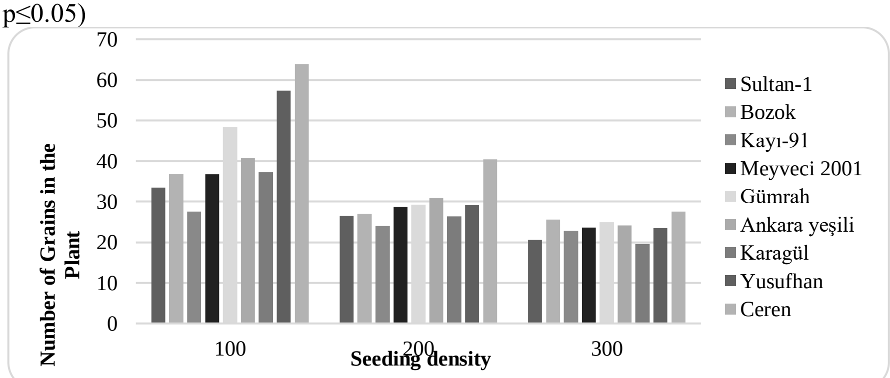

Figure 4. Effect of Seeding Density X Variety Interaction on the Number of Seeds in the Plant

Seed Yield per Plant (g): The effect of three different seeding densities on the seed weight of nine different green lentil varieties planted in summer under Kırşehir ecological conditions was investigated. In this study, the effect of seeding density, variety and seeding density X variety interactions on seed weight in the plant was determined to be statistically significant ($p<0.01$)

Table 8. Seed Yield per Plant (g) in Green Lentil Varieties of Different Seeding Densities

Variety	Seeding Density (seed per m^2)							
	100		200		300		Ort.	
Sultan-1	1.57	c-f	1.11	gh	1.03	gh	1.23	bc
Bozok	1.85	c	1.70	cd	1.39	d-g	1.65	a
Kayı-91	1.63	c-e	1.15	f-h	0.93	h	1.24	c
Meyveci 2001	1.93	bc	1.24	e-h	1.24	e-h	1.47	ab
Gümrah	2.28	ab	1.25	e-h	1.26	e-h	1.60	a
Ankara yeşili	1.73	cd	0.93	h	0.98	gh	1.21	c
Karagül	2.31	ab	1.12	gh	1.11	gh	1.51	a
Yusufhan	2.49	a	1.07	gh	0.94	h	1.50	a
Ceren	1.36	d-g	0.90	h	0.92	h	1.06	c
Averages	1.90	a	1.16	b	1.09	b		

*The difference between means marked with the same letter is insignificant (Duncan, $p\leq0.05$)

It has been determined that the effect of seeding density on seed yield per plant is important, and the highest average seed yield per plant is 1.90 g. was obtained from a seeding density of 100 seeds/m2. The seeding density with the lowest average seed yield per plant is 1.09 g. became 300 seeds/m2. The application where the sowing density is 200 seeds per square meter is 1.160 g. It can be seen in Table 8. that it has an average seed yield per plant of . Although the number of branches and branching in the plant are affected by environmental conditions and agricultural practices such as plant density, this is a variety characteristic. Krarup (1984) stated that as the density increases, the number of branches on the plant decreases and therefore plant productivity decreases. As the density decreases, the number of branches on the plant increases, and accordingly the number of pods and plant productivity increase; stated that yield was positively related to a high number of pods and branches. In fact, another researcher, Idris (2008), stated that increasing the plant habitat, in other words, reducing the seeding density, increases the number of pods per plant and ultimately gives the highest seed yield.

When the seed yield per plant is evaluated on the basis of varieties, the average seed yield per plant is 1.06 g. with 1.65 g. It was found to vary between . Statistically, the fact that the effects of varieties on seed yield per plant is at the level of 1% shows that the difference in seed yield per plant between some varieties is very high. The highest average seed yield per plant is 1.65 g. While Bozok lentil variety had the lowest average seed yield per plant, 1.06 g. It was determined that it was in the Ceren lentil variety (Table 8). Among the other lentil varieties tested, Yusufhan, Gümrah and Karagül lentil varieties were found to be in the same group with Bozok lentil variety in terms of the highest average seed yield per plant. Other lentil varieties were found to follow these four varieties in terms of average seed yield per plant. It has been found that varieties with high pod count and thousand seed weight also have high seed yield per plant. In many studies, it has been found that seed yield per plant has a significant effect on the number of primary branches, flowers and pods of the plant and plant height (Jain et al. 1991, Mikhov et al. 1987). Stoilova (1998) evaluated the morphological, phenological and agronomic characteristics of 120 lentil materials in a two-year study in Bulgaria. They found significant genetic differences between genotypes in terms of the examined characters. In the study, the seed weight of the materials was 0.27-2.2 g. They reported that it varies between .

When the interaction relationship between seeding density and variety in terms of plant seed weights is examined, the highest seed weight in the plant is 2.49 g per 100 seeds/m². While it was detected in Yusufhan lentil variety, the variety with the lowest seed weight per plant was 0.92 g at a seeding density of 300 seeds/m² and Ceren lentil variety were determined. This shows that each variety responds differently to different seeding densities.

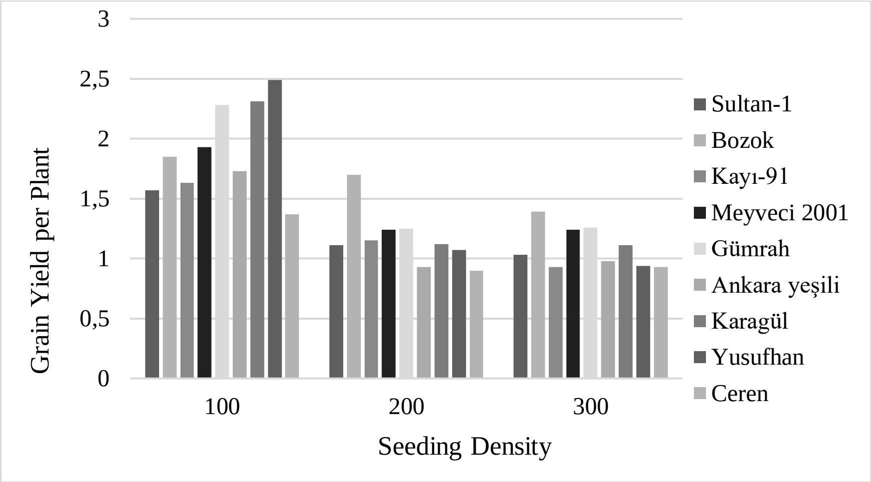

Figure 5. Effect of Sowing Density X Variety Interaction on Seed Yield per Plant

Seed Yield: It was determined that the effect of seeding density and variety factor on seed yield was statistically significant ($p<0.01$). (Table 3). At the same time, it was observed in the study that the seeding density variety X interaction was significant ($P\leq0.05$) (Table 3).

When the results regarding sowing density were evaluated, it was determined that the seed yield averages varied between 157.4 and 204.9 kg/da. It is seen in Table 9. that increases in seed yield are detected as seeding density increases and the highest seed yield is obtained from the most frequently planted application. In other words, the highest value was reached with a seed yield of 204.9 kg/da at a seeding density of 300 seeds/m^2. However, in the experiment, at the sparsest seeding density of 100 seeds/m^2, the lowest seed yield was reached at 157.4 kg/da. In the research, it was determined that the 200 seeds/m^2 sowing density, which is in the middle of these two seed densities applied with frequent and infrequent sowing, gave a medium seed yield with a seed yield of 177.3 kg/da.

An increase in seed yield was achieved in this study due to the increase in sowing density. Siddique et al. (1998) found that it would be appropriate to apply higher seeding rates in places where growing conditions are unfavorable and individual plant growth is limited. Akbar and Morteza (2008) reported that the highest seed yield in winter sowing was obtained at

200 seeds/m^2 at 806 kg/ha, whereas in spring sowing, the seed yield was obtained at 583 kg/ha at a seeding density of 400 seeds/m^2.

In this study, where the highest seed yield was obtained from a seeding density of 300 plants/m² and the lowest seed yield was obtained from a seeding density of 100 plants/m², the effect of seeding density was statistically significant. Another researcher, Slinkard (1976), reported that the highest seed yield was achieved at a seeding density of 129.2 plants/m². Mckenzie et al. (1985) from a seeding density of 200 plants/m²; Orhan et al. (1986) from a seeding density of 250 plants/m²; Ali Khan and Kiehn (1989) from the seeding density of 100 plants/m²; Pawloski and Bujak (1989) from a seeding density of 200 plants/m²; Shoaib (1992) from a seeding density of 400 plants/m²; Tanyolaç (1992) reported a seeding density of 400 plants/m², and Ağsakallı and Olgun (1999) reported that they obtained the highest seed yield from a seeding density of 300 plants/m². Bozoğlu and Pekşen (1997), Karadavut et al. (2001) reported that seed yield increases as seeding density increases.

Table 9. Seed Yield in Green Lentil Varieties of Different Seeding Densities

Variety	Seeding Density (seed per m^2)							
	100		200		300		Ort.	
Sultan-1	139.2	j *	152.3	h-j	171.0	e-ı	154.1	d
Bozok	166.3	f-j	165.8	f-j	203.0	b-d	178.3	c
Kayı-91	143.0	h-j	173.1	d-h	217.7	a-c	177.9	c
Meyveci 2001	138.4	j	188.5	c-g	216.9	a-c	181.2	bc
Gümrah	158.1	g-j	201.1	b-e	230.9	ab	196.6	ab
Ankara yeşili	145.1	H-j	171.9	d-h	191.0	c-f	169.3	cd
Karagül	190.0	c-f	195.7	c-f	244.9	a	210.2	a
Yusufhan	196.9	c-f	198.4	c-e	203.0	b-d	199.4	a
Ceren	140.3	ıj	149.4	h-j	166.4	f-j	152.0	d
Averages	157.4	c	177.3	b	204.9	a		

*The difference between means marked with the same letter is insignificant (Duncan, $p \leq 0.05$)

Additionally, Kantar et al. (1994) reported that both seed and total yield increased depending on the increasing sowing dose, however, there was a slight increase in seed yield after the 8.5 kg/da sowing norm. Turk et al. (2003), the highest and lowest pod number values were obtained from 80 and 120 plants/m². They stated that the decrease in the number of pods at 80 plants/m² may be due to the increase in competition between plants for growth factors such as the number of branches. They reported that yield is directly related to plant density and that as plant density increases, yield will also increase. They reported that they obtained the highest yield from 120 plants/m², and that the increase in yield observed as the density increased was due to the presence of many plants with more pods per unit area. They reported that although the effect of the increase in productivity per unit area was due to the high number of pods per unit area, the increased plant density did not affect the number of pods. Köse et al. (2017) found that seed yield increases as the number of plants per unit area increases.

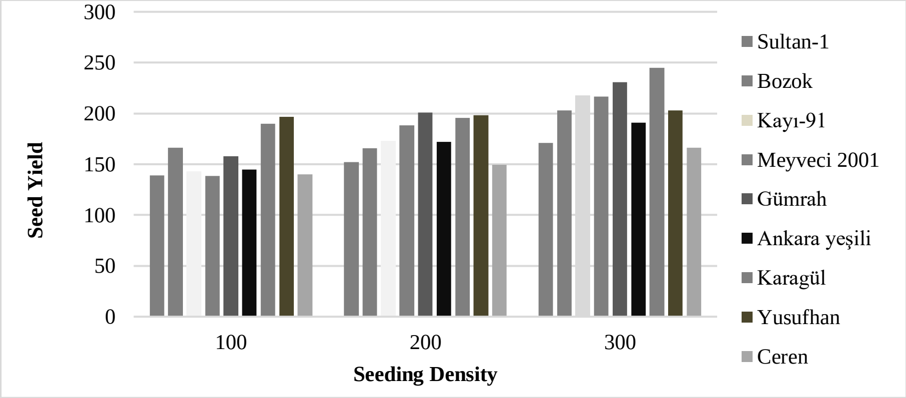

Figure 6. Effect of Sowing Density X Variety Interaction on Seed Yield

When the study results were evaluated on a variety basis, seed yield varied between 152.0-210.2 kg/da due to the statistically significant difference in seed yield between varieties. In the study, the highest seed yield average was found in Karagül lentil variety with 210.2. Table 4.30 shows that Yusufhan variety with 199.4 kg/da is among the high seed yield varieties in the research. In addition to these high-yield varieties, there are also varieties with lower seed yield than these varieties. It was determined that among the varieties with lower seed yield compared to these high-yield varieties, the Ceren variety had the lowest seed yield with 152.0 kg/da. Seed yield, which is the result of the interaction of many factors affecting the variety and variety, has a complex structure. Apart from variety characteristics, seed yield may vary depending on cultivation technique and ecological conditions.

Kose et al. (2017) in Yozgat, the average yield per decare of 9 lentil genotypes planted at different densities was; It has been reported that it varies between 112.0 and 200.6 kg/da. It was also reported that the highest yield was obtained from the Bozok variety, while Gümrah and Karagül lentil varieties were in the same statistical group.

5. CONCLUSION

There are not enough regional studies on green lentils in our country. In this study, which was conducted to close this gap, it was once again seen that studies on the determination of cultural practices such as plant density are not likely to lose their currency due to changing climate and soil conditions. It has been determined that changes in climatic factors in particular affect the data. In addition to selecting varieties for each region, other agricultural practices must also be determined for those varieties. In addition to the selection of productive varieties for this region, seeding density is one of the most important agricultural practices. Among agricultural practices, seeding density is one of the important issues of cultivation technique. In addition to many factors such as climate conditions, sowing time and seed size are factors that affect the sowing density. For this reason, determining the seeding densities of different lentil varieties is an important issue.

The finding that the sowing density x variety interaction was significant in the study shows that varieties respond differently to different sowing densities. It was determined that the Karagül variety had the highest seed yield in the average of three different sowing densities, Yusufhan variety with the highest seed yield at 100 seeds/m² with 196.9 kg/da, Gümrah variety with 201.1 kg/da seed yield at 200 seeds/m² and 300 seeds. At the /m² seeding density, it was determined that the Karagül variety gave the highest seed yield with 244.9 kg/da. In this case, it was concluded that Yusufhan and Karagül varieties were suitable for sparse seedings, while Gümrah and Karagül varieties were more suitable for dense seedings. We believe that Karagül lentil variety is more suitable for frequent or sparse seeding than other varieties (Yağmur, 2023)

6. REFERENCES

Ağsakallı, A., Olgun, M., 1999, Kırmızı mercimek Malazgirt-89 çeşidinde en uygun ekim sıklığı tespiti, *Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi*, 9 (1): 31-43.

Akbar, M.A., Morteza, A.Z., 2008, Study on effects of Seed Rate and Sowing Date on Lentil seed yield in North Khorasan dryland condition *Khorasan Agricultural and Natural, Resources Research Center*,36.

Akdağ, C., 1996, *Yemeklik Tane Baklagiller*. Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Yayınları. Tokat.

Alı-Khan, S.T., Kiehn, F.A. 1989, Effect of Date and Rate of Seeding, Row Spacing and Fertilization on Lentil. *Can. J. Plant Sci.***,** 69: 377-381.

Anonim. 2016. http://arastirma.tarim.gov.tr/tarlabitkileri.

Aydoğan, A., Aydın, N., Karagöz, A., Karagül, V., Horan, A., Gürbüz, A., 2003. *İç Anadolu ve Kuzey Geçit Bölgelerindeki Yeşil Mercimek (Lens culinaris* Medic.*) Genetik Kaynaklarının Toplanması, Karakterizasyonu ve Ön Değerlendirmesi.* Türkiye 5. Tarla Bitkileri Kongresi, 13-17 Ekim 160-165.

Biçer, BT., Şakar, D. 2011, Mercimek (*Lens Culinaris* Medik.) Hatlarının Verim ve Verim Özellikleri Yönünden Değerlendirilmesi. *Hr.Ü.Z.F. Dergisi*, 15 (3): 21-2.

Borah, U.K. 1996, Response of lentil genotypes to seed rates under rainfed conditions. *J. Agric. Sci. Soc. North-East India*. 9(1): 92.

Bozdemir, Ç. 2007, *Yazlık yeşil mercimek hatlarının Ankara ekolojisinde performanslarının belirlenmesi*. Yüksek Lisans, Tezi Selçuk Üniversitesi, Fen Bilimleri Enstitüsü.

Bozoğlu, H., Pekşen, E. 1997, *Farklı Sıra Arası Mesafelerinin Mercimeğin Tane Verimi ve Bazı Agronomik Özellikleri Üzerine Etkileri*. Türkiye II. Tarla Bitkileri Kongresi, 595-597, Samsun.

Canbolat, M. 2014, *Kahramanmaraş Koşullarında Değişik Mercimek (Lens Culinaris* medic.*) Çeşitlerinde Ekim Sıklığının Verim ve Verim Unsurları Üzerine Bir Araştırma*. Yüksek Lisans Tezi, Kahramanmaraş Sütçü İmam Üniversitesi, Fen Bilimleri Enstitüsü.

Coşkuner, Y., Karababa, E. 1998, *T*ürkiye'de Mercimek Üretim Potansiyeli ve İşleme Teknolojisi, *Gıda*. 23(3), 201-209.

Demir, I (2013) Oilseed crop cultivation in TR71 region and effects of climate change. Turkish Journal of Agriculture-Food Science and Technology 1, 73–78.

Demir, İ. (2020 a). Inter and intra row competition effects on growth and yield components of sunflower (*Helianthus annuus* L.) Under rainfed conditions. *JAPS: Journal of Animal & Plant Sciences, 30*(1).

Demir, I. (2020 b). Improving seed and oil yield of sunflower (Helianthus annuus L.) by using different inter and intra row space combinations. *J. Glob. Innov. Agric. Soc. Sci*, *8*, 147-153.

Demir, İ. (2020 c). Comparing the performances of sunflower hybrids in semi-arid condition. *Türk Tarım ve Doğa Bilimleri Dergisi*, *7*(4), 1108-1115.

Demir, I. (2021). The evaluation of confectionery sunflower (*Helianthus annuus* L.) cultivars and populations for yield and yield components. *International Journal of Agriculture, Environment and Bioresearch*, *6*(01), 179-186.

Erksine, W., Nassib, A.M. ve Telaye, A. 1988. *Breeding For Morphological Traits. World Crops: Cool Season Food Legumes.* Editor: R.J. Summerfield. Kluwer Academic Publishers. Pp: 117. The Netherlands.

Idris, A.Y. 2008, Effect of seed size and plant spacing on yield and yield components of faba bean (*Vicia faba* L.). *Res. J. Agrc. & Biol. Sci*., 4 (2): 146-148.

Jain, S.K., Sharma, H.L., Mehra, R.B., Khare, J.P. 1991, Multiple Correlation and Regression Analysis in Lentil. *Lens.* 18 (1-2):11-13.

Kantar, F., Çağlar, Ö., Akten, Ş. Erzurum Şartlarında Ekim Sıklığının Mercimeğin (*Lens Culinaris* Medic.) Verimine Etkisi. *Atatürk Ü. Zir. Fak.Dergisi***,** 25(3): 390-397.

Karadavut, U., Erdoğan, C., Özdemir, S., Şener, O. 2001, *Ekim Sıklığının Mercimekte (Lens culinaris* Medic.*) Verim ve Verim Kriterlerine Etkisi*. Türkiye IV. Tarla Bitkileri Kongresi, 385-390, Tekirdağ.

Köse, Ö.D.E., Bozoğlu, H., Mut, Z. 2017, Yozgat Koşullarında Yetiştirilen Yeşil Mercimek Genotiplerinin Verimine Ekim Sıklığının Etkisi. *KSÜ Doğa Bil. Dergisi.*, 20: 351-355.

Krarup, AH. 1984, The Effects of Sowing Dates and Rates on Lentil Yield Components. *Lens* 11(1):120-135

Mckenzie, B., Hill, G.D., White, J.G.H. 1985, The Effect of Sowing Date and Population on Yield of Lentils, Field Crop Abstracts, 41(12), 9065.

Mckenzie, B.A., Hill, G.D., White, J.G.H., Meijer, G., Sikken, G., Nieuwenhuyse, A., Kausar, A.G. 1986, *The Effect of Sowing Date and Population on Yield of Lentils (Lens culinaris* Medic.*)*. Proceedings, Agronomy Society o New Zeland. 16: 29-33; 10, New Zeland.

Mikhov, M.I., Stoeva, I., Ivanov, P., Mihov, M.I. 1987, *Biological and Economic Characters of Regionally Released and Promising Lentil Varieties in Bulgaria*. Plant Breeding Abstracts, 057-08532.

Orhan, A., Aydın, H., İnci, D. 1986 *Mercimek* (*Lens culinaris* Medic.)*'te Ekim Sıklığı ve Sıra Aralığının Verime Etkisi*. Güneydoğu Tarımsal Araştırma Enstitüsü Müdürlüğü Gelişme Raporları (Yayınlanmamış).

Pawlowski, F., Bujak, K. 1989, *Effect of Row Spacing and Seeding Rate on the Yield of Lentil* (*Lens culinaris* Medic.*)*. Famietnih. Pulawski, 95:157-167.

Sepetoğlu, H. 2002, *Yemeklik Dane Baklagiller Ders Kitabı*, Ege Üniversitesi Ziraat Fakültesi Yayınları, İzmir, 262.

Shoaib, Y.O. 1992, *Effects of Sowing Dates and Seeding Rate on Lentil in Eastern Libya*. Lens Newsletter, 19 (2): 21-22.

Siddique, K.H.M., Loss, S.P., Regan, K.L., *Pritchard, D.L., 1998,* Adaptation of lentil (*Lens culinaris* Medik.) to short season Mediterranean-type environments: response to sowing rates *Australian Journal of Agricultural Research* 49 (7): 1057-1066.

Slinkard, A.E. 1976, *Lentil Seeding Rate Studies in Saskatchewan. Lens*. Lentil Experimental New Service, 3:32-33.

Stoilova, T., Pererıra, M. G., 1999. Morphological Characterization of 120 Lentil (*Lens culinaris* Medik.) *Accessions. Lens Newsletter*, 26: 7-9.

Tantekin, M., Anlarsal, AE. 2008, *Diyarbakır Ekolojik Koşullarında Bazı Kışlık Kırmızı Mercimek (Lens culinaris Medic.) Çeşitlerinde Farklı Ekim Sıklıklarının Verim ve Verim İle İlgili Özelliklere Etkisi Üzerine Bir Araştırma,* Çukurova Üniversitesi Fen Bilimleri Enstitüsü, 19, 5.

Tanyolaç, B. 1992, *Van Ekolojik Koşullarında Mercimeğin Çeşit ve Adaptasyon Özellikleri Üzerinde Araştırmalar*. Yüksek Lisans Tezi Ege Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Anabilim Dalı, 51, İzmir.

Turk, MA.,Tawaha, AM., El-Shatnewi, MKJ. 2003, Response of Lentil (Lens culinaris Medik) to Plant Density, Sowing Date, Application in the Absence of Moisture Stres *J. Agronomy & Crop Science* 189: 1-6.

Yagmur M, D Kaydan 2004. Effects of sowing densities and phosphorus doses on some phonologic, morphologic characters and seed yield of dry bean under irrigation condition in Van, Turkey. Pakistan Journal of Biological Sciences 7 (10), 1782-1787.

Yağmur M, D Kaydan, 2005. Mercimek (Lens culinaris Medik.)'te yapraktan gübrelemenin tane verimi ile bazı verim özelliklerine etkisi Yuzuncu Yıl University Journal of Agricultural Sciences 15 (1), 31-37

Yağmur M, M Engin, 2005. Nohut (cicer arietinum l.)'ta fosfor ve azot dozları ile bakteri (rhizobium ciceri) aşılamanın bazı morfolojik özellikler ile tane verimi üzerine etkileri ve bazı bitkisel ….Yuzuncu Yıl University Journal of Agricultural Sciences 15 (2), 103-112

Yagmur M, D Arpali, F Gulser, 2017. Effects of zinc and urea as foliar application on nutritional properties and grain yield in barley (Hordeum vulgare l. Conv. Distichon) under semi-arid conditionFresen. Environ. Bull 26, 6085-92

Yağmur M, 2023. Effects of Seeding Rates and Sowing Times on Grain Yield and Yield Components in Rye (Scale cereale L.) Under Dry Condition.Manas Journal of Agriculture Veterinary and Life Sciences 13 (1), 9-16

Zulkadiroğlu, G. Çölkesen, M. İdikut, L. Çokkızgın, A. Girgel, Ü. Tanrıkulu, A. Canbolat, M. Güneş, M. 2015, Kahramanmaraş Koşullarında Farklı Mercimek (*Lens culinaris* Medik.) Genotiplerinde Bitki Sıklığının Verim ve Verim Unsurlarına Etkisinin Araştırması. *Harran Tarım ve Gıda Bilimleri Dergisi*, 19(3): 135-143.

CHAPTER 4

ROSEHIP: IS IT A MIRACLE FOR HEALTH?

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1. Introduction

Rosa canina is a species with great potential for distribution in Türkiye's forests. For this reason, rosehip is of great economic, ecological and social importance in Turkey. Its fruits contain bioactive components, many of which are known as phytochemicals, such as ascorbic acid, flavonoids, dimethyl sulfur, protein, tannin, Na, K, P, Mn, Mg, C, A, B, K, P vitamins, flavones, malic acid, pectin, stronelol. Besides, its seeds contain vanillin. It is known that the fruits are used to calm the blood, strengthen the blood, relieve whooping cough, cold, cough and pain, prevent cardiovascular diseases, cancer, prevent gastritis and ulcers, against diabetes, and the leaves are used against constipation and malaria. Its fruit is dried and used as tea. Its flowers and fruits are used in making jam, syrup, marmalade and liqueur. There is also information that a type of medicine used in the treatment of rabies was used in the past.

The great physician Ibn Sina (980-1037 AD) mentioned Rosa as alighol-kalb in his book "The Law of Medicine" and stated that it could cure ulcers, including mouth ulcer, and give strength to the gums and the body (Sharafkandi 2008). With these properties, it is considered the most easily accessible medicinal plant in rural areas in terms of public health (Yücel et al. 2019). In addition, it is known as the most effective remedy against hemorrhoids and diabetes mellitus in Turkish folk medicine (Orhan et al. 2009). Additionally, the roots and leaves of the plant have also been used against bronchitis (Fujita et al. 1995).

Rosa is very popular in traditional medicine. Boiled fruits are used as a diuretic and strengthener. When consumed with honey, Rosa juice can act as a diaphoretic and relieve colds, hypertension and liver disorders. Local people of Central Asia make jam from wild rose petals and use it as a heart strengthening and sedative. Boiled Rosa galls are used in the treatment of stomach ulcers, duodenal ulcers, malaria and pulmonary tuberculosis (Sokolov et al. 1977). Boiled rosehip treats stomach cancer. Boiled hips helps eliminate hemorrhoids, soothes pain and burning.

Hippocrates used rosehip in the treatment of gallbladder disease. Dioscorides also used it for abdominal pain. In Chinese traditional medicine, Rosa roots are used as an anthelmintic. In Tibetan traditional medicine, Rosa flowers are used to treat neurasthenia, atherosclerosis and tuberculosis. In

Mongolian traditional medicine, Rosa is used to treat headaches, dizziness, skin burning. In Bulgarian folk medicine, fruits and Rosa flowers are used as a diuretic and sedative (Jordanov et al. 1972; Tolekova et al. 2020).

2. Botanical Classification and General Herbal Evaluations

Rosehip is known as the fruit of the *Rosa canina* L., which belongs to the *Rosacea* family. The color of the fruit varies from red to orange, and approximately 71% of the fruit consists of pericarp and 29% seeds. Its average weight is between 1.25 and 3.25 g. Its fruit; It is rich in bioactive compounds such as vitamin C, carotenoids, tocopherol, phenolic acid, bioflavonoids, tannin, pectin, organic acids, amino acid, essential oil and unsaturated fatty acids. Additionally, rosehip is considered an alternative source of lycopene, containing 2.9-35.2 mg of per 100 grams fruit. Therefore, it can be used as effective antioxidants because it contains significant amounts of phenolic compounds (Yilmaz and Ercişli 2011). Dog rose, or *Rosa canina* L., grows wild over large areas in Europe, North Africa and Western Asia.

The genus *Rosa* includes approximately 100 species that are widespread in Europe, the Middle East, Asia and North America (Nilsson 1997) (Figure 1). It is a perennial, deciduous shrub with a thorny, thin stem, varying between 2-3 meters in height. The colors of its flowers vary from light pink to dark pink and white. The flowers are 4-6 centimeters in diameter and turn into red orange fruits when ripe. Rosehip, which is particularly well adapted to poor, dry and rocky soils and drought, can be propagated by many different methods (such as seeds, bottom shoots, cuttings, dipping and tissue culture).

Figure 1. Distribution area of wildrose on different contries (Tolekova et. al. 2020).

The name "dog rose" is its oldest name, as there are records that it was used in the treatment of dog bites for the first time in history. In addition, Avicenna (980-1037 AD), one of the oldest and most famous physicians in history, mentions *Rosa canina* L. as alighol-kalp in his work "The Canon of Medicine" and states that it can heal ulcer, including mouth ulcer. It states that it can strengthen the gums. Rosehip, an important source of vitamin C, is an effective medicinal plant used in the treatment of gingivitis and swollen or bleeding gums, which are the main clinical symptoms of scurvy (vitamin C deficiency) (Tolekova et al. 2020; Khazaei et al. 2020).

3. Considerations for Traditional Medical Use

As it is known, since vegetables and fruits are an important source of phytochemicals and other bioactive compounds, they are also considered as a serious source of therapeutic products that can prevent, alleviate or cure many diseases. Reactive Oxygen Species (ROS) play an important role in the development of these diseases. Sometimes the presence of ROS in the organism is beneficial because they are used in the immune response to kill extracellular bacteria. However, ROS can also contribute to undesirable effects because they trigger oxidation processes. When free radicals are produced in a living organism, many antioxidants act to protect the organism from oxidative damage.

The organism has three lines of defense in combating oxidative stress. The first line of defense consists of peroxidases and metal-binding proteins and prevents the formation of ROS. Secondly, it has been stated that polyphenols obtained from *R. canina* fruit have anti-cancer properties. This may also be due to the abundance of the carotenoid lycopene. Finally, the organism's third line of defense consists of enzymes that repair lipids, proteins, sugars and DNA from oxidative damage (Okuda 2005; Gupta et. al. 2012; Charles 2013) .

It is naturally found in vegetables, herbs, fruits, spices, tea, coffee and cocoa. Different epidemiological studies have shown that this plant-based distribution of disorders can reduce the risk of chronic disease and mortality disorders (Ebrhardt et al. 200; Ganesan et al. 2011).

Nowadays, more importance is given to herbal raw materials as a source of biological active substances. As a result of this great scientific interest, rosehip, a genus of wild plants belonging to the *Rosaceae* family, is widely used medicinally, as a source of vitamins and as a food raw material. The most valuable part of the fruit is known as pericarp and is used in products such as medicinal products, herbal teas, jam and marmalade. It is also used in probiotic drinks (Ahmad et. al. 2016).

Many studies have been conducted to examine the dynamics of vitamin accumulation depending on the forms and types of rose hips. In these studies, geographical location, meteorological conditions, soil, fertilizer application and other environmental factors create more or less differences in bioactive components (Tolekova et al. 2020). With increasing altitude of plants above sea level, the content of ascorbic acid, carotene, catechins, leukoanthocyanins, anthocyanins and flavonols increases, but the content of tannins in fruits decreases (Kotenko et al. 2011). It was determined that the species, change status and harvest time were important in the bioactive profile of rosehip. Ripe fruits contain high tocopherol and β-carotene, while mature fruits have high levels of ascorbic acid. This situation is supported by the literatures (Barros et al, 2011; Roman et al. 2013; Demir et al. 2014). At the same time, rosehip fruit as well as its leaves have a rich chemical composition. Leaves rank second in ascorbic acid content relative to the vegetative part of the plant. The presence of biologically active substances such as carotenoids, chlorophyll, tocopherols and flavonoids is evident in the leaves (Ghazghazi et al. 2010) (Fig.2).

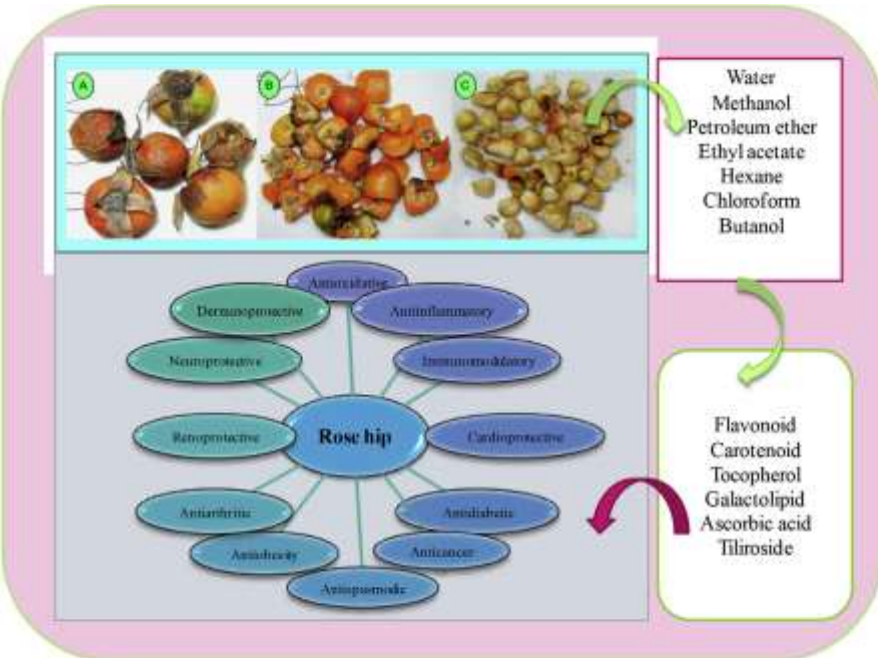


Fig. 2. (A) Ripe rose hips (B) Fragmented rose hips (C) The seeds. The solvents used for extraction of bioactive fractions, the phytochemicals and the biological benefits (Patel 2017).

Moreover; since *R. canina* has antioxidant properties, the possible protective effects of its fruits against the genotoxic effects of ethyl methanesulfonate were investigated by *Drosophila* wing somatic mutation and recombination test. Test results revealed the potential of *R. canina* as a natural genoprotective product (Kasimoğlu and Uysal 2016). A study on six plant species in 2010 year, including Rosa, collected from Southern Europe, showed that this plant has excellent antioxidant properties, so it can be used as an alternative to synthetic antioxidants (Egea et al. 2010).

Rosehip is rich in polyphenolic compounds such as proanthocyanidins and flavonoids such as quercetin and catechin (Türkben et al. 2010). It has been observed that the high phenolic and flavonoid content of rosehip is associated with antioxidant activity (Wenzig et al. 2008), and rosehip extract containing these phenolics shows significant antioxidant activity even when deprived of vitamin C (Daels-Rakotoarison et al. 2002) (Table 1). This activity includes protective effects against oxidative stress, increased activity of antioxidant enzymes such as superoxide dismutase and catalase, and protective effects on intercellular communication (Yoo et al. 2008).

Tablo 1. *Rosa* species, the phytochemicals in the rose hips and biological functions (Patel 2017).

Biological function	**Rosa species**	**Phytochemicals**
Antioxidative (Hepatoprotective, prevent schemic stroke, antidepressant, prevent kidney stone)	*R. laevigata* Michx *Rosa damascena* Mill. *R. canina*	Flavonoids Tiliroside
Immunomodulation and anti-inflammation (Prevent osteoarthritis and rheumatoid arthritis pain; protect gut mucosa)	*R. canina* *R. roxburghii* *R. multiflora* *R. moschata*	Triterpene acid Galactolipid Unsaturated fatty acids
Cardiovascular	*R. laevigata* Michx	Flavonoids
Anti-obesity and antidiabetic.	*R. canina* L.	Tiliroside
Anticancer and antimicrobial	*R. canina* *R. nutkana* *R. woodsii*	Polyphenols Phenolic contents Lycopene
Cosmetics	*R. canina*	Proanthocyanidins

Rosehip also has anti-inflammatory, anti-diabetic and anticancer effects. Much of the anti-inflammatory effect that rosehip has has been attributed to its high amounts of galactolipids, a class of compounds that have recently been shown to have antitumor-promoting and anti-inflammatory activity both in vitro and in vivo. Rosehip and its constituent galactolipids have also been found to inhibit the production of inflammatory mediators and provide chondroprotective effects in vitro. Unlike nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin, rosehip has anti-inflammatory effects that do not have ulcerogenic effects and do not inhibit platelets or affect the coagulation cascade or fibrinolysis, thus preventing potential side effects (Cohen 2012). In addition to having a high content of polyunsaturated fatty acids, R. canina has also been found to have significantly high antioxidant components (Kayahan et al. 2022).

Phenolic compounds and ascorbic acid found in rose hips provide health benefits. Ascorbic acid has positive effects on cognitive decline and Alzheimer's disease. Rosehip is also rich in vitamin C, carotenoids, tocopherol, bioflavonoids, tannins, essential oils and pectin. (Fig. 3 and 4) (Ercişli et al. 2007; Demir et al. 2014), these pseudo-fruits are It is thought that it can constitute an alternative antioxidant source for the food industry and can also be used for therapeutic purposes. The distinctive orange to red color of rose hips occurs as a result of various carotenoids. The most abundant of these are β-carotene and lycopene, followed by β-cryptoxanthin, rubixanthin, zeaxanthin, and lutein (Hodisan et al. 1997; Anderson et al. 2011).

Carotenoids and tocopherols are compounds important to human nutrition. Carotenoids can prevent some diseases and cancer by acting as provitamin A, while tocopherols have vitamin E activity and have the ability to scavenge free radicals. These compounds are natural antioxidants important for human health (Mayne 1996; Marmol et al. 2017). The antioxidant properties of tocopherols in rose hips are based on their ability to form stable, small reactive radicals as a result of the dissociation of a hydrogen atom from a hydroxyl group after interaction with active radicals (Smirnova et al. 2008).

Another important phytochemical in the species known as rosehip is quercetin. Quercetin is the most abundant flavonoid that forms glycosides with different sugar structures and accumulates in higher plants (Schieber et al. 2003). Quercetin and its derivative quercetin-3-O-glucuronide provide chemoprotection of mitochondrial function through antioxidative effects by preventing excessive production of reactive oxygen species (Guo et al. 2013). As a matter of fact, many researchers have found in their studies on Rosa species in different geographies that vitamin C, quercetin and ellagic acid are the most abundant phenolic compounds (Ercişli et al. 2007; Fuji and Saito 2009; Tumbas et al. 2012).

Rosehip seeds contain oil and minerals. The fatty acids in the seed are mainly represented by linoleic, oleic, linolenic, palmitic, stearic and arachidonic acid (Özcan, 2002). The amount of oil in the seeds varies between 5% and 18% depending on the species. In rosehip, 97% of the seed oil consists of linoleic, oleic, palmitic and stearic acid; The remaining 3% consists of 12 minority fatty acids (Novak 2005). The oils in its seeds are mainly used in the cosmetic industry and pharmaceutical industry. Its fruits increase the biosynthesis of collagen, stimulate the immune system, and increase body resistance against constant effort (Kılıçgun and Dehen, 2009; Orhan et al, 2009). It has been determined that rosehip oil components such as citrenellol, geraniol and nerol mediate antibacterial effects (Boskabady et al. 2011). In many studies conducted on mice with rosehip extracts, it has been reported that *R. canina* extract has anti-dementia properties and improves spatial learning and memory (Daneshmand et al. 2016).

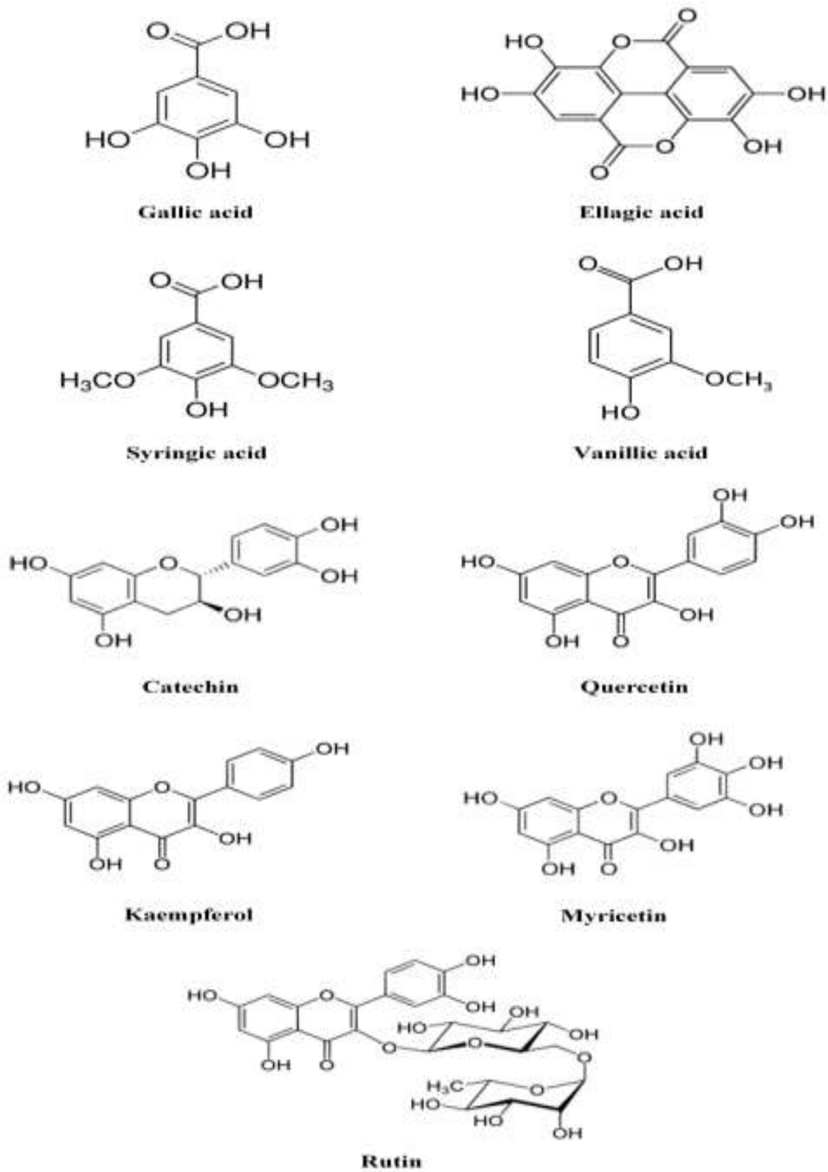
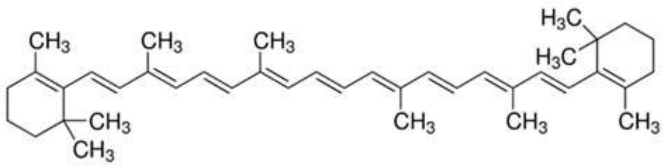
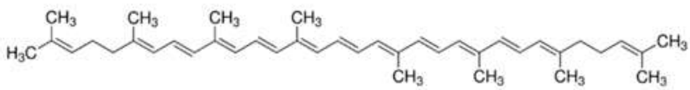


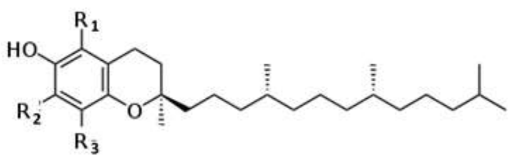
Figure 3. Chemical formulas of hydrosoluble phenolic compounds of rose hips.



β-Carotene



Lycopene



	R_1	R_2	R_3
α-Tocopherol	CH_3	CH_3	CH_3
β-Tocopherol	CH_3	H	CH_3
γ-Tocopherol	H	CH_3	CH_3
σ-Tocopherol	H	H	CH_3

Figure 4. Chemical formulas of lipid-soluble antioxidants of rose hips.

Reproductive system disorders have become one of the important health problems all over the world in recent years and their incidence is rapidly increasing. Synthetic drugs cause high costs and serious side effects. Research has shown that herbal products can be used effectively in the management and treatment of male and female reproductive disorders (Farzana et al. 2017). As a result of an in vivo study on the effects of *R. canina* extract on body and testicular weights, serum testosterone levels, number of germ cells and Sertoli cells, sperm parameters, average testicular seminiferous luminal and tubular and epithelial height in mice, it was determined that the extract had protective effects against reproductive toxicity. (Nowrouzi et al. 2019).

Rosehip may achieve promising results in cancer treatment. The antitumor effect of this fruit is related to its phytochemical composition, and studies conducted in different regions have yielded different results. It has been proven that *Rosa canina* has a stopping effect on cancer cells with its phenolic contents. (Thanan et al. 2014; Guimarães et al.2015).

Rosehip (Rosa canina L.) is prominently used in the prevention and treatment of rheumatoid arthritis (RA) (Kirkeskov et al. 2011). In particular, rosehip powder reduced symptoms associated with rheumatoid inflammation in clinical studies (Kharazmi and Winther 1999; Cohen 2012). Some phytochemicals found in plants provide positive effects in the treatment of osteoporosis. In related studies; The high antioxidant content of Rosa canina reduced the damage caused by excessive ROS in bone tissue and increased bone formation by stimulating collagen matrix synthesis and osteoblast differentiation (Devareddy et al.). 2008).

Rosa canina also has anti-aging activity. Positive results have been reported in many studies on this subject. These positive effects are related to their antioxidant content. So that; Some phytochemicals act by scavenging reactive oxygen species produced by UV radiation and thus reducing skin damage. Of these, vitamin C may have multiple effects in protecting the skin, as it plays a direct role in collagen formation along with its antioxidant effect. In addition, some components of *Rosa canina* may have a protective effect against UV-induced inflammation and damage, as they have an anti-inflammatory effect. Moreover; Antioxidant compounds and polyunsaturated fatty acids both prevent cell membrane damage This effect explains the effects of *Rosa canina* in extending the lifespan of red blood cells (Marmol et al. 2017).

Studies on the contents of rosehip roots are too limited. A study conducted on the roots of *R. canina* and *R. pimpinellifolia* species, traditionally consumed in the Eastern Anatolia Region of Turkey, has shown that rosehip root parts contain quite high levels of phenolic compounds. Although current studies have mostly focused on rosehip fruit, cyanidin has been detected in the roots of black rosehip (*Rosa pimpinellifolia* L). Indeed, this substance is a major component of the anthocyanin class of flavonoids. Unlike the fruit parts, phenolic compounds such as epicatechin, gallic acid, syringic acid, quercetin dihydrate and naringenin have been determined in the roots of *R. canina* and *R. pimpinellifolia*. It has been observed that catechin and its derivatives are concentrated in both the fruit and root parts of both species. In the study, it was observed that the roots of *R. pimpinellifolia* and *R. canina* contained higher amounts of phenolics compared to the fruits (Macit et. al. 2023).

In light of all the issues tried to be explained above; Due to all the phytochemical composition it contains, rosehip can be considered an interesting treatment option that should be considered for problems involving oxidative stress and/or pro-inflammatory state in humans.

3. CONCLUSION

Phenolic compounds and ascorbic acid stand out among the water-soluble phytochemicals found in rose hips. Studies have proven that rose hips have a higher vitamin C content than most edible fruits. Although rosehip extract cannot alleviate complex metabolic, autoimmune, and degenerative diseases, it can support the body's healthy redox state. The extracts have been confirmed to be beneficial against fatty liver, osteoarthritis, rheumatoid arthritis, obesity, cancer, kidney stones, depression, skin problems and other pathologies. Consumers' increasing use and demand for nutritional supplements today further increases the importance of this plant. Although potential functional food resources are being wasted before our eyes, food insecurity has become critical, especially in developing countries. However, it is true that additional research is needed on the differentiation of the contents of fruits, leaves, seeds and roots according to species, altitude, ecology and even ripening periods. Therefore, to ensure that rosehip consumption does not cause any adverse health effects, additional empirical analyzes regarding the nutritional profile of rosehips are needed.

REFERENCES

Ahmad, N. & Anwar, F. Rose hip (*Rosa canina* L.) oils. (2016). *Essential Oils in Food Preservation, Flavor and Safety*, pp. 667‑675. (Academic Press, 2016). https:// doi. org/ 10. 1016/ B978-0- 12- 416641- 7. 00076-6.

Andersson, U.; Henriksson, E.; Ström, K.; Alenfall, J.; Göransson, O.; Holm, C. (2011). Rose hip exerts antidiabeticeffec ts via a mechanism involving downregulation of the hepatic lipogenic program. Am. J. Physiol. Endocrinol. Metab., 300, E111–E121.

Boskabady, M. H., Shafei, M. N., Saberi, Z., & Amini, S. (2011). Pharmacological effects of rosa damascena. Iranian Journal of Basic Medical Sciences, 14(4), 295e307.

Charles, D.J. (2013).Antioxidant Properties of Spices, Herbs and Other Sources; Springer: New York, NY, USA.

Cohen M. (2012). Rosehip - an evidence based herbal medicine for inflammation and arthritis. Aust Fam Physician. Jul;41(7):495-8.

Daels-Rakotoarison DA, Gressier B, Trotin F, et al. (2002). Effects of *Rosa canina* fruit extract on neutrophil respiratory burst. Phytother Res;16:157–61.

Daneshmand P, Saliminejad K, Dehghan Shasaltaneh M, Kamali K, Riazi GH, Nazari R, Azimzadeh P, Khorram Khorshid HR. (2016). Neuroprotective effects of herbal extract (Rosa canina, Tanacetum vulgare and Urtica dioica) on rat model of sporadic Alzheimer's disease. Avicenna J Med Biotechnol 8: 120-125.

Demir, N.; Yildiz, O.; Alpaslan, M.; Hayaloglu, A. (2014). Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (Rosa L.) fruits in turkey. LWT Food Sci.Technol., 57, 126–133.

Devareddy, L.; Hooshmand, S.; Collins, J.K.; Lucas, E.A.; Chai, S.C.; Arjmandi, B.H. (2008). Blueberry prevents bone loss in ovariectomized rat model of postmenopausal osteoporosis. J. Nutr. Biochem., 19, 694–699.

Eberhardt, M.V.; Lee, C.Y.; Liu, R.H. (2000). Antioxidant activity of fresh apples. Nature, 405, 903–904.

Egea, I., Sánchez-Bel, P., Romojaro, F., Pretel, M.T. (2010). Six edible wild fruits as potential antioxidant additives or nutritional supplements. Plant Foods Hum Nutr., 65(2),121-129.

Ercisli, S.; Orhan, E.; Esitken, A. (2007). Fatty acid composition of Rosa species seeds in turkey. Chem. Nat. Compd., 43, 605–606

Farzana M, Yasir Khan M. (2017). Herbs act on female and male reproductive system: recent advances. World J Pharm Pharm Sci; 6: 1734-1747.

Fujii, T.; Saito, M. (2009). Inhibitory effect of quercetin isolated from rose hip (Rosa canina L.) against melanogenesis by mouse melanoma cells. Biosci. Biotechnol. Biochem., 73, 1989–1993.

Fujita T. , Sezik E. , Yesilada E. , Tabata M. , Yesilada E. , Honda G. , TakedaY., Tanaka T. and Takaishi Y. (1995).Traditional medicine in Turkey VII. Folk medicine in middle and west black sea regions . *Econ Bot.* 49 : 406 – 22

Ganesan, K.; Kumar, K.S.; Rao, P.S. (2011). Comparative assessment of antioxidant activity in three edible species of green seaweed, enteromorpha from okha, northwest coast of India. Innov. Food Sci. Emerg. Technol., 12, 73–78.

Ghazghazi, H., Miguel, M.G., Hasnaoui, B., Sebei, H., Ksontini, M., Figueiredo, A.C., Pedro, L.G., Barroso, J.G. (2010). Phenols, essential oils and carotenoids of Rosa canina from Tunisia and their antioxidant activities. African J. Biotechnology. 9(18), 2709-2716..

Guimarães, R.; Barros, L.; Calhelha, R.C.; Carvalho, A.M.; Queiroz, M.J.R.; Ferreira, I.C. (2014). Bioactivity of different enriched phenolic extracts of wild fruits from northeastern portugal: A comparative study. Plant. Foods Hum. Nutr. 69, 37–42.

Guo, X.D.; Zhang, D.Y.; Gao, X.J.; Parry, J.; Liu, K.; Liu, B.L.; Wang, M. (2013). Quercetin and quercetin-3-O-glucuronide are equally effective in ameliorating endothelial insulin resistance through inhibition of reactive oxygen species-associated inflammation. Mol. Nutr. Food Res., 57, 1037–1045

Gupta, S. C., Hevia, D., Patchva, S., Park, B., Koh,W., & Aggarwal, B. B. (2012). Upsides and downsides of reactive oxygen species for cancer: The roles of reactive oxygen species in tumorigenesis, prevention, and therapy. Antioxidants & Redox Signaling, 16(11), 1295e1322. http://doi.org/10.1089/ars.2011.4414.

Harrison, F.E. A (2012). Critical review of vitamin c for the prevention of age-related cognitive decline and Alzheimer's disease. J. Alzheimers Dis., 29, 711–726.

Hodisan, T.; Socaciu, C.; Ropan, I.; Neamtu, G. (1997). Carotenoid composition of *Rosa canina* fruits determined by thin-layer chromatography and high-performance liquid chromatography. J. Pharm. Biomed. Anal. 16, 521–528.

Jordanov D., Nikolov P., Boychinov A. (1972). Phytotherapy. Treatment with officinal herbs. Sofia, pp. 347.

Kayahan, S., Ozdemir, Y. & Gulbag, F. (2023). Functional Compounds and Antioxidant Activity of *Rosa* Species Grown In Turkey. *Erwerbs-Obstbau* **65**, 1079–1086 https://doi.org/10.1007/s10341-022-00688-5

Kasimoğlu C, Uysal H. (2016). Genoprotective effects of aqueous extracts of Rosa canina L fruits on ethyl methanesulfonate induced DNA damage in Drosophila melanogaster. Cumhuriyet Sci J; 37: 241-241.

Khazaei M, Khazaei M.R., Pazhouhi M. (2020). An Overview Therapeutic Potentials of Rosa canina: A Traditionally valuable herb. WCRJ 2020; 7: e1580.

Kharazmi, A.; Winther, K. (1999). Rose hip inhibits chemotaxis and chemiluminescence of human peripheral blood neutrophils in vitro and reduces certain inflammatory parameters in vivo. Inflammopharmacology, 7, 377–386.

Kilicgun H., Dehen A., (2009). In vitro antioxidant effect of *Rosa canina* in different antioxidant test systems. Pharmacognosy Research, 1: 417-420.

Kirkeskov, B.; Christensen, R.; Bügel, S.; Bliddal, H.; Danneskiold-Samsøe, B.; Christensen, L.P.; Andersen, J.R. (2011). The effects of rose hip (*Rosa canina*) on plasma antioxidative activity and c-reactive protein in patients with rheumatoid arthritis and normal controls: A prospective cohort study. Phytomedicine, 18, 953–958.

Kotenko, M.E., Guseinova, B.M., Daudova, T.I. (2011). Vliyanie lugovo lesnıch pocv Terskogo Sudakskoy nizmennosti i gornogo Dagestana na biochimiceskiy sostav shipovnika. Vestnik DGRU, Techniceskiye Nauki, 15, 177-182.

Macit, M, Aras, A, Capanoglu Guven, E, Bakir, S, 2023. Investigating the Content and Bioaccessibility of Phenolic Compounds In Roots of *Rosa canina* L. *and Rosa pimpinellifolia* L. Yuzuncu Yil University Journal of Agricultural Sciences, 33(2): 163-173. DOI:https://doi.org/10.29133/yyutbd.1231881

Mármol I, Sánchez-de-Diego C, Jiménez-Moreno N, Ancín-Azpilicueta C, Rodríguez-Yoldi MJ. (2017). Therapeutic Applications of Rose Hips from Different Rosa Species. Int J Mol Sci. May 25;18(6):1137.

Mayne, S.T. (1996) β-carotene, carotenoids, and disease prevention in humans. FASEB J., 10, 690–701.u

Nilsson, O.R. (1997). Flora of Turkey and the East Aegean Islands; University Press: Edinburgh, UK.

Nowak, R. (2005). Fatty acids composition in fruits of wild rose species. Acta Soc. Bot. Pol., 74, 3.

Nowrouzi F, Azadbakht M, Kalehoei E, M Modarresi. (2019). Protective effect of Rosa canina extract against doxorubicin-induced testicular toxicity in mice. Braz Arch Biol Technol; 62: e19180017.

Okuda, T. (2005). Systematics and health effects of chemically distinct tannins in medicinal plants. Phytochemistry, 66, 2012–2031

Orhan N, Aslan M, Hosbas S, Deliorman OD. (2009). Antidiabetic Effect and Antioxidant Potential of *Rosa canina* Fruits. Pharmacognosy Magazine;5 (20):309-315.

Ozcan M., (2002). Nutrient composition of rose (*Rosa canina* L.) seed and oils. Journal of Medicinal Food 5(3): 137–140.

Patel S (2017). Rose hip as an underutilized functional food: Evidence-based review, Trends in Food Science & Technology, 63:29-38.https://doi.org/10.1016/j.tifs.2017.03.001.

Roman, I., St_anil_a, A., & St_anil_a, S. (2013). Bioactive compounds and antioxidant activity of Rosa canina L. biotypes from spontaneous flora of Transylvania. Chemistry Central Journal, 7(1), 73. http://doi.org/10.1186/1752-153X-7-73.

Schieber, A.; Berardini, N.; Carle, R. (2003). Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. Cv.“Tommy Atkins”) peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. J. Agric. Food Chem., 51, 5006–5011.

Sharafkandi, A. (2008). The Persian Translation of Qanoun Fi Al-Tibb (Or the Canon of Medicine), Vol. 2. Soroush Press, Tehran, Iran.

Smirnova, V.A., Klimochkin, Yu.N. (2008). Vitamins and coenzymes: study guide. Izd. Gos. Tech. University Samara, Samar, pp. 91.

Sokolov, S. Ya., Svyazev, O. A., Kubli, V. A. (1977). Areas of trees and shrubs of the USSR. Taxaceae- Aristolochiacae. Leningrad, Science, 1, 164.

Teodorescu, A. A. *et al.* (2023). Customized technological designs to improve the traditional use of *Rosa canina* fruits in foods and ingredients. *Plants.* 12(4), 754. https:// doi. org/ 10. 3390/ plant s1204 0754.

Thanan, R.; Oikawa, S.; Hiraku, Y.; Ohnishi, S.; Ma, N.; Pinlaor, S.; Yongvanit, P.; Kawanishi, S.; Murata, M. (2014). Oxidative stress and its significant roles in neurodegenerative diseases and cancer. Int. J. Mol. Sci.,16, 193–217.

Tolekova, S., Sharmanov, T., Sınyavskıy, Y., Berzhanova, R., et al. (2020). Antioxidant, Pharmacological, Medical Properties and Chemical Content of Rosa L. Extracts. International Journal of Secondary Metabolite, 7(3), 200-212. https://doi.org/10.21448/ijsm.726140

Tumbas, V.T.; Canadanovic-Brunet, J.M.; Cetojevic-Simin, D.D.; Cetkovic, G.S.; Ethilas, S.M.; Gille, L. (2012). Effect of rosehip (*Rosa canina* L.) phytochemicals on stable free radicals and human cancer cells. J. Sci. Food Agric., 92, 1273–1281.

Türkben C, Uylaser V, Incedayi B, Celikkol I. (2010). Effects of different maturity periods and processes on nutritional components of rosehip (*Rosa canina* L.). Journal of Food, Agriculture and Environment;8:26–30.

Wenzig EM, Widowitz U, Kunert O, et al. (2008). Phytochemical composition and in vitro pharmacological activity of two rosehip (*Rosa canina* L.) preparations. Phytomedicine;15:826–35

Yilmaz, S.O., Ercisli, S. (2011). Antibacterial and antioxidant activity of fruits of some rose species from Turkey, Romanian Biotechnological Letters, 16, 6407-6411.

Yoo KM, Lee CH, Lee H, Moon BK, Lee CY. (2008). Relative antioxidant and cytoprotective activities of common herbs. Food Chemistry;106:929–36.

Yücel E, Karakaya T, Yücel D (2019). Importance of Rosa canina's In Terms Of Public Health And Turkey Forestry. International Journal of

Environmental Research and Technology E-ISSN:2667-4041 2(3): 204-208, 2019 Proceeding Book of 3th IPSAT Congress.

CHAPTER 5

TROPHIC STATE INDICES: THE USE IN LAKE MANAGEMENT, FISHERIES AND AQUACULTURE

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1. Introduction

In aquatic sciences, trophy refers to the qualitative (verbal) and quantitative (numerical) nutrient richness and biological productivity of the water source, in other words, its productivity. Trophic status (TSI) or level indices (TLI) have been developed and used for lakes, dam lakes, reservoirs, large ponds and wetlands. They are useful methods for assessing ecological health and productivity of the lakes. Lake trophic state (TS) classifications provide information about the lake ecosystems' condition and can be used as indicator of ecosystem services (e.g., aquaculture and fisheries, drinking and swimming water, recreational opportunities, and aesthetics) and harmful events in the lakes (cyanobacteria dominance).

TS knowledge of waters is an invaluable for lake managers, researchers, fish farmers and other stakeholders. TS is an indicator for lake productivity, water quality, biological integrity, and fulfillment of designated use criteria (legislations, certifications etc.) Recreation, habitat and species diversity, natural heritages and ecological values, fisheries and aquaculture are closely related to lake water quality. So, water quality monitoring is integral to lake management, eutrophication control and fisheries of lakes. The USA's Clean Water Act and New Zealand governmental office require all lakes of the country be classified according to TS. TS can be used in public communication, and a management instrument to provide the scientific base of eutrophication and lake character.

The TSIs are used to understand nutrient levels, biological productivity and general TS, and for the management and rehabilitation of water resources for drinking water, fisheries, aquaculture, recreation, eutrophication control. *Trophy indices* (TSI and TLI) also used as *eutrophication indices* (EI) for monitoring eutrophication of water bodies. This section mainly covers the Trophic Status Index (TSI), Trophic Level Index (TLI), Turkey Surface Water Quality Trophic Level Index (TrTLI) OECD trophic classes, and TRIX.

The formulas and their parameters used by related indices, their temporal and spatial changes, their relevance to trophic state, calculation methods, misunderstandings and misclassifications or failures in determinations, and their use in water resources, fisheries and aquaculture management are presented and evaluated in this chapter. Yet, there is not an internationally defined and accepted TSI but majority of countries use TSI and TLI. Special attentions were focused to Turkey’s water bodies and legislations (laws and related directives) and applications relating to trophic indices.

2.Trophic state: principles and historical development

2.1. What is trophic state?

Lake classification is determination of the present status of the water body. The classification stated “the point of water’s occupied place in terms of morphology of water (lake, dam lake, reservoir, wetland etc.), shallow or deep, nutrient rich or poor, turbid or clear, salty or freshwater, glacial or tectonic, mountain or low land, natural or man-made etc.”.

Trophic state (TS) classification is to classify of the current status of a waterbody’s in its productivity (all organic matter that produced inside). Limnologists, fisheries biologist, environment and lake managers, aquaculture experts give special attention to TS. TS classification often related with *“water quality”* “*fish productivity*” “*pollution/eutrophication trend*” “*water availability for different use*” and the issues and problems about lake management, and rehabilitation efforts.

TS term and concept was used by E. Naumann referencing to lakes in year 1929. TS mean the “quantitative production of phytoplankton or algal biomass” due to the impacts on a lake’s biological properties. TS refers to phytoplankton growth rate in a special period (productivity) or weight or biomass (production). The measurement of biomass is easier relatively and compatible directly into the management issues of lakes, biomass is accepted as a trophic indicator and used as a parameter. Therefore, TS has been formulated with various indices mainly based on biomass. Carlson trophic state index TSI (published in 1977), a well-known and widely accepted one. TSI based regression equations between the parameters and complying classification scale. Some other TS indices were developed, majority of them have same logical basis of Carlson’s. But they vary considerably in their classification approach, variable selection and category numbers.

2.2. Eutrophication of water bodies and the consequences

In lake ecosystems, biotic/living and non-living/abiotic factors interact with each other. The basic expressor of trophic state, algal growth, relies on nutrient uptake from water. Algal biomass constitutes the main component of primary production in water bodies, having a basic role in trophic status. Eutrophication, enrichment of plant nutrients, excessive algal growth and its consequences is getting more and more significant problems to aquatic ecosystems and important threats for aquatic ecosystem health, integrity and water quality worldwide. By developing the eutrophication aquatic ecosystems losses their biodiversity, degrades their integration, harmful algal blooms raises, oxygen deficiency (hypoxia) occurs in hypolimnion (over the lake bottom).

Increase of nutritive elements accelerates algal growth consequently, biomass increases. Additionally, niches of harmful algae species (blue-greens) altered by the severe eutrophication, and they dominate to other algal populations. This ecologic succession can upset the balance among aquatic organisms, and results in water quality degradation and lost economic (fisheries, recreation, drinking water supply etc.) value of the water body.

Algae overgrowth on the lake epilimnion makes shading effect, increase turbidity and inhibit sun rays to penetrate deeper layers. These poor light conditions suppress phytoplankton growth, decrease of overall lake productivity, and in consequence fish yields in lakes. After the death and decaying of algal biomass causes oxygen depletion, toxic gas formation (NH_3, H_2S), delivering algal toxins that harmful for human, fish and other aquatic animals. All these events decline water quality and limits water use for different purposes. The development of eutrophication process can be measured and monitored effectively and precisely by trophic state indices quantitatively. The obtained TSI can be transferred to management decisions, the measures that will be undertaken and to measure of lake rehabilitation success. Eutrophication caused by human activities (agriculture, animal production , aquaculture, industrial and domestic effluents etc.) are causal factors on TS. To quantify and manage human impacts on TS, it requires knowledge on level and variability TSIs of lakes. Setting management objectives and targets also should be integrated with TSI.

3. Trophic state indices, parameters, determination and use

3.1. Carlson Trophic State Index (TSI)

The TSI was developed by Robert G. Carlson as a new approach lake trophic classification in 1977 based on the data taken Minnesota lakes of N. America and classified, further detailed and concluded in 1996 and 2007. TSI is most commonly used index to evaluate trophic state of lakes. TSI uses Chlorophyll-a (Chl μg/L) and total phosphorus concentrations (TP μg/L) and Secchi Disc depth measurements (SD m.) of a lake, it can be calculated any of this parameters. TSI supplies a numeric value that correspond to trophic state of water body in mean of stated parameter and is rated on a scale from “0” to “100” for most lakes, higher value corresponds to higher trophy (Table 1 and 2). Traditional systems divide the trophy continuity into three classes: oligotrophic, mesotrophic, and eutrophic, but there is often no clear delineation of these divisions. TSI can be estimated separately for SD, Chl, TP by using following original equations or simplified forms proposed by Carlson (Table 1). The better indicators for TS may differ from one lake to another and of course seasonally. Thus better indicators should be chosen related to the lake’s condition and use. Besides the first developer (Carlson) of TSI method stated as separately evaluation of TSI by different parameter. Some authors proposed to take the average of three values as trophic state of the water body as CTSI= [TSI(SD)+TSI(CHL)+TSI(TP)/3. If Nitrogen is also effective and a limiting factor on trophic state of water body, or it is needed the TSI(TN) index added also to trophic state estimations.

Practically, **oligotrophic** (TSI score 0–40) the water body has little biological productivity, "good" water quality for different water use; **mesotrophic** (TSI 40–60), has a moderate level productivity, "fair" water quality ; and **eutrophic** to **hypereutrophic** (TSI 60–100), has the highest productivity but "poor" water quality. Trophic Quotient (TQ) has close principle to TSI, and base the ratio of Chl-a to TP concentration. It provides a simple way to assess the trophic status of a lake based on the relationship between nutrient levels and algal biomass. But three is no useful formulas and classification for TQ yet.

Table 1. Trophic state indices (Carlson, 1977, Kratzer & Brezonik 1981, Carlson, 2007)

Trophic indices	Trophic indices (simplified)
$TSI(SD) = 10\left(6 - \frac{\ln SD}{\ln 2}\right)$	TSI(SD) = 60 - 14.41 ln(SD)
$TSI(Chl) = 10\left(6 - \frac{2.04 - 0.68 \ln Chl}{\ln 2}\right)$	TSI(CHL) = 9.81 ln(CHL) + 30.6
$TSI(TP) = 10\left(6 - \frac{\ln \frac{48}{TP}}{\ln 2}\right)$	TSI(TP) = 14.42 ln(TP) + 4.15
SD (m), Chl/CHL (µg/L), TP (µg/L), TN (mg/L) ln: natural logarithm	Kratzer & Brezonik TN Index TSI(TN) = 54.45 + 14.43 ln(TN)

Table. 2. Trophic State Index, chlorophyll-a, phosphorus, Secchi disc depth, and water body's trophic classes (Carlson & Simpson 1996)

Trophic State Index	Chlorophyll (µg/L)	Phosphorus (µg/L)	Secchi disc depth (m)	Trophic Class
<30—40	0—2.6	0—12	> 8—4	Oligotrophic or hypo trophic
40—50	2.6—7.3	12—24	4—2	Mesotrophic
50—70	7.3—56	24—96	2—0.5	Eutrophic
70—100+	56—155+	96—384+	0.5—<0.25	Hypertrophic

3.1.1. The parameters of TSI

Chlorophyll-a (Chl-a)

As the basic primer productivity component, phytoplankton biomass can be measured by several manners: chlorophyll (specific one, Chl-a), algal bio volume, dry or organic weights and ATP. Each of them contribute valuable information for biomass estimating, but they also ignore some biomass forms, or include some interferences. Chl-a is regarded as an important indicator solely, thus Chl-a has become the most popular estimator of the biomass. Chl-a has advantages, such as it can measure a specific characteristic of plant biomass not found in bacteria (bacteria is also significant fraction of carbon weight in water). Chl-a is found in live algae, but isolating and measuring of it is difficult as accurately. Common spectrophotometric test measures Chl-a with some weakness as the method also includes other chlorophylls and degradation pigments, thus Chl-a might be overestimated, and the amount of Chl-a in algae cell can vary considerably also. Chl-a is strongly controlled by nutrient concentrations in temperate lakes (including Turkey's lakes). High Chl-a concentration indicate high phytoplankton density in water, thus there is a logarithmic relation between biomas and Chl-a. Therefore Chl-a is considered as biological indicators and parameters or resulted/effect parameter for TSIs.

Total Phosphor (TP)

Phosphor is known a limiting (minimum) factor for terrestrial and aquatic plant life for a long time. The amount of algal biomass, proliferation and growth depend on P concentration and availability. The algae cells use up primarily orthophosphate ion (reactive P) as P source. Since all P forms convert to reactive P easily, total P (TP) is used safely to estimate TS and eutrophication. TP is considered as physio-chemical or cause parameters in eutrophication and trophic state indices. TSI formula was derived by using the relationship between TP and *summer* Chl-a concentration defined by Dillon and Rigler (1974). Therefore TSI estimation should be based on summer samplings.

Total Nitrogen (TP)

TP constitute all kind of nitrogen that used up by plant sells directly (NO_3, NH_4) or indirectly after biochemical hydrolysis, oxidations reduction and mobilization of N bearing molecules (proteins, amino acids, nucleic acids etc.) Scientists usually use TN to estimate trophic state/level indices of lakes because; N is an essential plant nutrient (for live algal biomass) and limiting nutrient in some water bodies, and in many lakes the availability of N can limit primary productivity. N is an eutrophication indicator that elevated levels of TN can indicate eutrophication, where excessive nutrient loads lead to increased algal blooms and subsequent negative effects on water quality. And N is a complementary to P, while TP is often a primary indicator in TSI calculations, incorporating TN provides a more comprehensive assessment of nutrient status and potential algal growth. TN concentration, typically reported in mg/L in some trophy index as µg/L (eg. OECD Trophic class).

Secchi Disk Depth/light visibility

Secchi disk depth (SD) is a measure of vertical light penetration in a lake and relative amount of turbidity. Usually the increase nutrients and algal biomass, as consequence, limits light penetration in water and SD visibility decrease, inversely correlated with SD. Besides the algal biomass richness, water color and other suspended organic and inorganic particles (detritus, bacteria, clays) effects SD visibility. Studies on water clarity were indicated also that TS was dependent, and an important extent, defined by the level of phytoplankton colonization. Significant correlations were found between SD and lake's TS, therefore SD is an integral monitor of TS and used in all TSIs. Secchi disk (SD) depth, estimates the density of algal particles, but includes non-algal particles also thus when measure SD depth special attention should be given the sources of turbidity (lower SD value) . Turbidity values are also affected by the shape of the particles, instruments that used and the water color. SD is often lower in shallow lakes due to reduced clarity caused by wind driven resuspension of sediments (organic detritus and inorganic particles, such as clays). SD is considered as physico-chemical or cause parameters in eutrophication and trophic state indices.

3.1.2. Estimation of TSI

Where no single parameter can measure algal biomass without problems, it needs an objective method for TS estimation. The first attempts include examining a list that characteristics associated with 3-4 (or 5) trophic classes (oligotrophic, mesotrophic, eutrophic, hypereutrophic etc.). Thereafter designating the state fitting with the trophic category of actual lake's trophic characteristics is made. In verbal definition of lakes we use this still, but this kind of classification not useful for management purposes because we don't use this data to manage the lake; whether an activity can disturb the lake's original situation, cause pollution or eutrophication, or drinking water quality by removing only color and turbidity. For management we need quantitative facts and figures that we can alter, mitigate, rehabilitate etc.

As a quantitative method for TS, R. Carlson developed a numerical index of TSI based regression relationships between Chl-a and SD (Carlson 1977). Because it is not measuring algal biomass, only estimating Chl-a as the estimator of the biomass, it is called as "index". Chl-a was chosen since it is specific to algae and can measure algal abundance even in the presence of non-algal particles. TSI give the possibility of a greater sensitivity to change in TS. Each 10 units on the scale represent a halving or doubling of SD, a biomass measure that is directly relevant to communication with the public.

Other additional trophic parameters are incorporated by coupling variables to SD or Chl-a by regression models, and give the opportunity to use the variables (TP and TN) other than Chl-a (Figure 1.). parameters provide their own information that may be missing from the others. Therefore, the more variables measured, the more information we can gather about TS. TP and TN are causal parameters also (cause or produce algal biomass) and link loading estimates of nutrients to TS. Following steps are proposed by Carlson (2007) to estimate TSI.

1. It should be looked for reasons why a Chl-a value might not reflect biomass values in chosen index period (season, zooplankton grazing, algaecide spraying, harvesting, high macrophyte density). Be aware of Chl-a value and its changes to spatial-temporal scale (in winter few algal biomass, in summer might be huge, different lake section have different algae densities) and what the driven factors of algal biomass.

2. TSI for Chl-a can be calculated by using the formula (Table 1.) .

3. If you want to estimate only TSI-Chl-a, and you don't interested more about the lake's situation that's all. If you don't have Chl-a values;

4. Calculations are made of TSI using related formulas of SD, TP, or TN (Table 1.). But be aware of that they contain more potential errors comparing to Chl-a's.

5. The differences should be compared between TSIs calculated by variables. Where the variation seems random that's OK (Figure 1.). Are there systematic and repetitive deviations? If yes, ask the error sources or repeat the estimation by increased sampling frequency in spatial and temporal scale after the new data obtained follow the above steps.

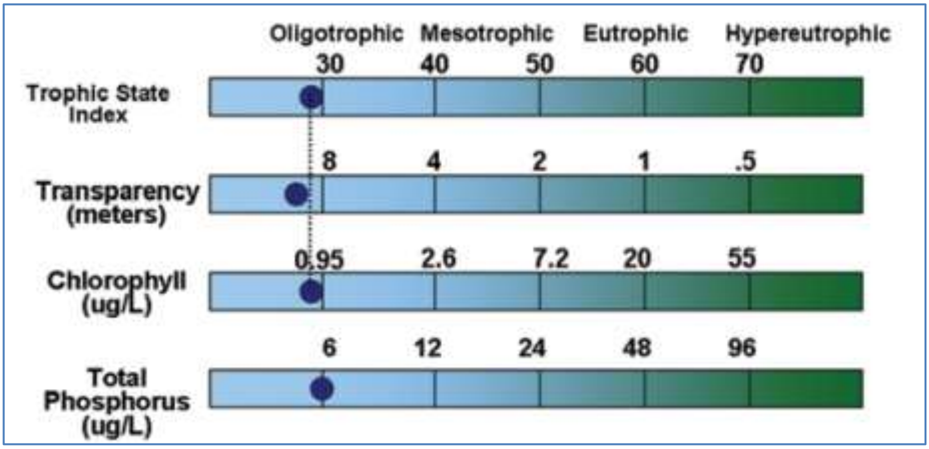

Figure 1. Parameter values, TSI scores and trophic classification of Clearwater Lake, Minnesota, USA. (Carlson, 2007). (Estimations are based on seasonal averages; all tree parameters produce similar TSI)

3.1.3. The confusions and difficulties in TSI estimations

- If any index score looks like match the lake's situation contrary to exception, Two factors contribute to this; low correlation between the variables since these indices are derived from regression between the variables and in case natural variation may be happen so correlations are rarely perfect. And second factor relates to lake's origin and type (primary production limiting TP, TN or both, lake type, altered heavily, man-made, karstic, low land mountain, transition water etc.).

- Even under the best conditions a variation of ±5 or more of TSI units might be possible. For this reason, if for no other failures occur or detect, multiple sampling and more data are necessary throughout the lake and over time.
- It is a well-known fact that water quality parameters of a lake may be change even in few hours (a storm, heavy rainfall, effective bottom current and waves, overturn). In similar case, Chl-a should be used as the primary index since it is a direct estimator of algal weight (and a effect parameter for primary production).
- TP and SD interfere each other and not solely related (a bottom storm and spring/autumn overturns carry excessive TP to upper lake section and it increase unusually level etc.).
- TSI was developed under low aquatic macrophyte conditions. If the lake covered a dense macrophyte (as in case shallow lakes and some wetlands of Turkey that important bird areas and some considered the Ramsar sites) and resulting very low algal growth the index might be underestimated (lake is considered as oligotrophic). Even the trophy level high, because of low Chl-a and clear appearance of water (highly transparent, deeper SD) the TSI value will be very low. The presence dense macrophyte biomass can produce a misclassification. TSI can work acceptable well in the presence of macrophyte in larger lakes/dam lakes in limited lake area and macrophyte constitute a small proportion of the total biomass and (with exception of completely free-floaters), rooted macrophytes derive most of their nutrients from sediments. Therefore macrophyte biomass is not significantly correlated with in lake present and external nutrient loading to the lake. In such circumstances, indices (reflect the direct impact) may misclassify the trophic state. But indices might be a good indicator of the impact of external nutrient loading. Macrophyte rich lakes have considerable amounts of biomass but are rarely considered in trophic classifications. So, dense macrophytes and clear water conditions are future research topic in TS quantifications.
- TP and SD depth should be not used solely to determine trophic state only if Chl-a values are not available. Because they interfere

each other and weakly related to algal biomass. If water color and non-algal particulates significantly affect transparency, SD has limited value as a TS indicator (with easily wind driven turbidity in shallow lakes, dam lakes that loads very much erosion driven turbidity etc.).

- Chl-a can be suppressed in fast water flow-through situations (in dam lakes with apparent water flows to lower course) and where zooplankton grazing has temporarily cleared the water.
- If original regression formulas constructed from glacial origin lakes, and algal biomass limited mainly by TP (TN:TP ratio ca. 30:1) this can cause unexpected scores.
- In many colored, shallow, and wind-stirred water bodies, dam lakes and reservoirs (load heavily runoffs that carry soil erosion's derived clays) a reasonable TSI cannot be estimated. Because in that cases, non-algal turbidity and unusual water color affect the variables. Non-algal particles (clays) affect TP and SD indices, causing systematic deviations of TSI (TP) and TSI (SD) indices and basically color lowered markedly the transparency.
- In these instances, index should be calculated for 2-3 variables and a general trend is taken as trophic state class with averaging the scores (CTSI= [TSI(SD)+TSI(TP)+TSI(CHL-A]/3).
- Besides of trophic indices other characteristics of water bodies (macrophyte density and coverage in shallow lakes, oxygen depletion and hypolimnetic anoxia etc. Usually indicate eutrophic condition) should be considered also.

3.1.4. The Use of TSI for Management purposes

TS estimation is the first step in water body management. Usually TS is determined by land use and geology, they also affect the biota of the lake. In this frame if we can estimate TS, also can back calculate the amount of loading to the lake, and forward to predict the impact algal status on the remainder of the biota. Verbal and numerical TS have been constructed to predict fish yield, fish community composition, hypolimnetic oxygen depletion, and sedimentation rates. So, TS stands as a pivot variable in lake management.

Desired TSI values differ between water body users, managers, protection of water and water pollution control. A fisherman who catch fish from a lake want of his water moderate trophic level. Because lower TSI means few fish in there; higher TSI means a nuisance of smaller sized and lower priced (raw) fish, moderate level of TSI can support middle and sized fish that preferred by market and consumer willing. But water-fowl, duck hunters prefer higher TSI scored eutrophic, aquatic macrophyte covered, rich food suppliers of water bodies since there are many water birds can populate there. The residents, that live the shore of a lake or reservoir, pool to be oligotrophic, as they are more pleasant for swimming, boating and other water sports. A municipality wants its drinking water source should be ultra-oligotrophic since good and preferred water quality to drink and use households beside cost effectiveness water supplying for domestic needs. A fish farmer who raises fish in net cages strongly needs good quality lake water for his fish welfare and health. Natural resource management bodies of a country are responsible for reconciling these conflicting uses and determining what a water body's trophic index should be.

3.2. Trophic Level Index

Trophic Level Index (TLI) is used to characterize the ecological health of lakes and dam lakes based on nutrient levels and algae growth, helps estimate biological productivity in freshwater ecosystems. TLI provides an integrated measure of water quality by considering nutrients and algae. It includes the parameters chlorophyll-a concentration, total phosphorus, nitrogen levels and transparency as Secchi disc depth. TLI values are calculated based on empirical relationships between these parameters and trophic status. It is presented as seven classification categories (range from ultra-micro trophic to hypertrophic; as numerical varies 0 to 7) the lower the score the better the condition of the lake and water quality (Table. 3). TLI is an analog of Carlson’s TSI, and was modified and formulated by Burns et al. (2000). It is proposed and used in New Zealand’s (NZ) inland waters, both indices use same parameters (SD, TP, Chl-a), additionally TLI includes total nitrogen (TN). This means that TN, in addition to TP, usually limits phytoplankton biomass in NZ lakes, N limitation seemingly more prevalent in NZ. NZ's legislation requires as objectives to be set for three of the four constituent TLI variables in the lakes. TLI's variables are highly inter-correlated, although the relationships between variables vary among lakes depending on lake characteristics.

Table 3. The lake types, trophic levels, parameter value limits of Trophic Level Index (Burns et. al., 2000)

Lake type	Trophic level	Chl-a (mg m^{-3})	Secchi depth (m)	TP (mg P m^{-3})	TN (mg N m^{-3})
Ultra-microtrophic	0-1	0.13-0.33	31-24	0.84-1.8	16-34
Microtrophic	1-2	0.33-0.82	24-15	1.8-4.1	34-73
Oligotrophic	2-3	0.82-2.0	15-7.8	4.1-9.0	73-157
Mesotrophic	3-4	2.0-5.0	7.8-3.6	9.0-20	157-337
Eutrophic	4-5	5.0-12	3.6-1.6	20-43	337-725
Supertrophic	5-6	12-31	1.6-0.7	43-96	725-1558
Hypertrophic	6-7	>31	<0.7	>96	>1558

Each TLI (Tli) is a logarithmic function connecting the trophic level to 4 "trophic" parameters. (Burns et al., 2000).

- $TLI_i = a_i + b_i * \log(Par_i)$
- TLI_i :indices (each of 4 parameters),
- a and b are coefficients, log: logarithm in base 10
- Par_i is SD depth, concentrations of TN, TP and Chl-a

Simplified verbal definition of TLI categories relation to lake characteristic are as follows:

- **Ultra-microtrophic -microtrophic (TLI 0-2)** lakes are very clean, high transparent (high SDD value), crystal blue waters with very low levels of N,P and algae, their origin commonly glacial and they located on mountainous areas.
- **Oligotrophic (TLI >2 -3)** lake is clear and blue in colour, with low levels N, P and algae

- **Mesotrophic (TLI >3-4)** lake has moderate level of N,P and algae
- **Eutrophic (TLI>4-5)** lake is murky greenish with high amounts of N, P and algae
- **Supertrophic-hypertrophic (TL >5)** lake has very high P, N, and can be overly fertile and often very low water clarity (low SD), excessive algae growth, recreational quality often very poor

3.2.1. Calculation of the TLI

The TLI is calculated by using annual averages of Chl-a, SD, TN, TP. (Burns et al., 2000):

TLI (Chl-a) = 2.22 + 2.54 *log(Chl-a)

TLI (SD) = 5.10 + 2.27 *log(1/SD–- 1/40)

TLI (TP) = 0.218 + 2.92 *log(TP)

TLI (TN) = -3.61 + 3.01 *log(TN)

TLI = [(TLI (Chl-a) + TLI (SD) + TLI (TP) + TLI (TN)]/4

3.2.2. Application of TLIs

Fisheries management: TLI helps the assessment the trophic state of lakes, informs us for decisions relating to nutrient management, fish and other aquatic organism stocking, and habitat restoration for better and sustainable fisheries. By tracking TLI over time, managers can monitor also changes in water quality.

3.3. The Trophic Level Index (TrTLI) of Turkish Surface Water Quality Directive

The legislative framework of trophic level index (TrTLI) in Turkish inland, coastal and transition waters lined in year 2004 by “Turkish surface waters quality directive (TrSWQD)”. The directive was ruled first as Water Pollution Control Directive, after several emendations its name adopted as TrSWQD (MoAF, 2012). The TrSWQD aims to determine the biological, chemical, physicochemical and hydro morphological qualities of surface waters, coastal and transitional waters, to classify them, to monitor water quality and quantity, to determine the purposes of use of these waters in connection with sustainable development goals. The directive take into account the balance of protection and use, and to determine the procedures and principles for the measures to be taken to protect and achieve good water status (*Art.Nr. 1*).

3.3.1. Estimation of Trophic Level Index (TrTLI)

Trophic levels of lakes, reservoirs and dam lakes are determined according to the classification given in Table 4. (*Table 9 in Annex-6.*) The analysis results of SD depth, chlorophyll-a, total nitrogen and total phosphorus parameters are taken as basis in the classification. The trophic level index value is calculated according to the equations. The final trophic level value is determined by taking the average of the index values calculated for the four parameters in the equations (*Art. Nr. 14(2*).

TLI(SD) = 60 - 14.41*ln(SD)

TLI(CHL-A) = 9.81*ln(CHL-A) + 30.6

TLI(TP) = 14.42*ln(TP) + 4.15

TLI(TN) = 54,45 + 14,43*ln(TN)

TrTLI(Average)= [TLI(SD) + TLI(CHL-A)+ TLI(TP)+ TLI(TN)]/4

SD: Secchi Disc Depth (m), CHL-A: Chlorophyll-a (µg/L)

TP: Total Phosphorus (µg/L), TN Total Nitrogen (mg/L), ln: natural logarithm

If a water body has more than one $TrTLI_i$ (2-4) and scores close to each other's the average level will be taken into consideration; If a water body has more than one $TrTLI_i$ (2-4) and scores are different the most higher TLI value be taken into consideration (Table. 4 and 5).

3.3.2. Applications of TrTLI in Türkiye

Fish farms cannot be established in lakes, dam lakes and reservoirs where drinking and utility water is supplied. However, in dam lakes (with wider surface area, such as Atatürk, Keban, Karakaya dam lakes) where economic zones are established by the General Directorate of State Hydraulic Works (SHW or DSİ), aquaculture and fishing may be permitted within the framework of the application principles determined jointly with the Ministry of Agriculture and Forestry(MoAF), provided with approval of Ministry of

Environment and Urbanization and Climatic Change (MoEUCC). In dam lakes where the lake area at the maximum water level is larger than 75,000 ha, aquaculture may be permitted in an area up to 0.1% of the area at the minimum water level. If permitted in dam lakes aquaculture cannot be carried out in areas closer than 1000 m to the drinking water intake structure and in bays where these drinking water abstraction structures are located (*Art. 14 (3)*). In dam lakes, surface area less than 75.000 ha, fish farming facilities are allowed to be established in an area of up to 3% of the water surface area at the minimum water level, with the approval of MoAF (*Art. Nr. 14(4))***.**

Existing or new established net cage fish farms shall continue aquaculture in dam lakes or reservoirs where water circulation is easily provided, in accordance to the trophic level, assimilation capacity and ecological status determined by MoAF. The analysis of TLI parameters shall be carried out by the farm owners and sent to MoAF. The analysis results shall be evaluated by the MoAF whether or not comply to the TLI scores (Table 4 and 5) (*Annex-6, Table 9 (Art. Nr. 14(5)*) After fish farm start operating, the analysis of TrTLI parameters shall be carried out by farm owner and results be submitted regularly basis to MoAF (*Art. Nr. 14(6)) The trophic states* of some water bodies, different origin and use summarized in Table 6 and some examples of lakes/dam lakes were given in Figure 2.

Table 4. Eutrophication criteria of lakes, dam lakes and reservoirs of Türkiye (Annex 6: Table 9. Amanded, OG-16/6/2021-31513; MoAF 2012)

Trophic level index value averaged (TLI)		Trophic level
>62		Hypertrophic
62		Eutrophic
60*	52	Mezotrophic
44		Oligotrophic
≤ 29		Ultraoligotrophic

* valid for reservoirs and dam lakes

Table 5. Trophic level limits for the parameters (Annex 6. Table. 10. **MoAF, 2012**)

Trophic levels	TP (µg/L)	TN (µg/L)	Chl- *a* (µg/L)	SD depth (m)
Oligotrophic	≤10	≤350	<3.5	>4
Mesotrophic	10>TP≥30	350>TN≥650	3.5-9.0	4-2
Eutrophic	30>TP≥100	650>TN≥1200	9.1-25.0	1.9-1
Hypertrophic	>100	>1200	>25.0	<1

3.3.2.1. Assessing the environmental impacts on surface waters

The TLI can be used for environmental impact assessment (EIA) on inland, coastal and transitional waters. To do this the waters are divided into categories as artificial, heavily modified and natural water bodies because the impacts will be varied among to water types. Namely, natural water are more resistant to environmental impacts but artificial water bodies have limited ecologic self-safety mechanisms. The location and boundaries of rivers, lakes, coastal and transitional waters are defined with their coordinates. Geochemical, geological and topographic features of the water body are determined in relation to its bottom/bed structures. The water and land ecosystems interactions are determined. The environmental impacts to water bodies are assessed quantitatively by using TrTLI scores and their variation in time and spatial scale in categories of point and nonpoint pollution sources, distribution, hydro morphologic, water use (irrigation, effluents, solid waste disposal, fisheries, aquaculture and other human activities). In the case of new project that rely on, production or usage before and after, determination of TrTLI's of water bodies could be supplied rational quantitative evidence for EIA.

Table 6. Trophic states of some Turkish water bodies estimated by trophic state indices

Water body, water quality, use, statues	Indices	Parameters	Trophic state	References
Acı Lake (Konya), brackish, salty	TSI	Chl-a, TP, TN, SD	oligotrophic	Akköz et al., 2009
Lake Meke (Konya) brackish water, wetlands, bird area, Ramsar site, ecotourism	TSI	Chl-a, TP, TN, SD	eutrophic	Akköz et al., 2009
Lake Suğla (Konya) Freshwater, irrigation, aquaculture, fisheries	TSI	Chl-a, TP, TN, SD	eutrophic	Akköz et al., 2009
Küçükçekmece Lagoon Lake (İstanbul), brackish, fisheries, wetland	TSI	TP, Chl-a, SD,	eutrophic	Aydın et. al., 2022
Sücüllü dam lake (Isparta), freshwater, irrigation, aquaculture	TSI	TP, Chl-a, SD,	oligotrophic (Chl-a), eutrophic (TP,SD)	Aslantürk & Çetinkaya 2022
Eğirdir Lake (Isparta), freshwater, drinking, irrigation, fisheries, recreation	TSI, TrTLI, OECD	TP, Chl-a, SD,	mesotrophic	Bulut and Kubilay, 20
Karkamış dam lake	TSI,	TP, Chl-a,	mesotrophic	Tepe et. al.,

(Gaziantep) freshwater, irrigation, fisheries, aquaculture	OECD	SD,TN		2018
Borçka dam lake (Artvin), irrigation, hydropover, aquaculture	TSI, TLI, TrTLI	TP, Chl-a, SD,TN	mesotrophic, non-algal turbidity,	Bilgin, 2020
Uzunçayır dam lake (Tunceli), freshwater, irrigation, aquaculture, hydropover, recreation	TSI	TP, Chl-a, SD,TN	Oligotrophic, N:TP<10 biomass limited by TN	Kutlu et. al. 2017

Eğirdir lake, Isparta, mesotrophic

Küçükçekmece lagoon lake, İstanbul, eutrophic

Gölcük crater lake, Isparta, oligotrophic

Salda lake, Burdur, mesotropic,

Suğla Lake, Konya, eutrophic

Borçka dam lake, Artvin, mesotrophic, trout farm

Figure 2. Some Turkish lakes/dam lakes in different trophic states and rainbow trout fish farms (M. Ceylan and https://images.app.goo.gl/bNzFN56add3jQcct9)

3.2.1.1. Protection Zones in Surface Waters

Some vulnerable areas (protection zones) as follows need more strictly considered to protect and manage; Areas allocated for use drinking water supply, allocated for the protection of economically important aquatic species (for fisheries and biodiversity), water bodies used for recreational purposes (including swimming), areas designated as sensitive in terms of nutrients within the scope of the “regulation on the protection of waters against pollution from agricultural sources (shortly nitrate regulation) and the regulation on urban wastewater treatment, areas allocated for the protection of habitats or species where the maintenance or improvement of water status is an important factor and the Natura 2000 areas. The trophic states of all those areas have major importance and their past, present and future of TP, TN, SDD, Chl-a values and corresponding numerical equivalent should be determined by TrTLI.

3.4. The OECD Trophic State Classes

The OECD developed global trophic state criteria in 1982 by undertaking a survey to classify lakes using any appropriate criteria by participation of limnologists and water quality experts from all of world. OECD criteria on trophic classification of lakes were quantified by relationships between Chl-a, SD, TP and TN, and probability scores (averages) were produced. TSs were constructed as eutrophic, oligotrophic or mesotrophic in respect to the ranges of the parameter values (Table. 7). The quantified relationship between nutrient load and trophic reaction in waters using Vollenweider's general model and derived systematic conclusions from the data, reaching globally accepted conclusions. The OECD criteria are used in some countries (including Türkiye) and by different authors to establish TS of a water body.

Table 7. The OECD's trophic state classification criteria (OECD, 1982)

Trophic level	TP (µg/L)	TN (µg/L)	Chl-a (µg/L)	Max Chl-a (µg/L)	Secchi Disc Depth (m)
oligotrophic	8	661	1,7	4,2	9,9
mesotrophic	26,7	753	4,7	16,1	4-2
eutrophic	84,4	1875	14,3	42,6	2,45

3.5. Eutrophication Index (TRIX)

This index focuses specifically on the eutrophication process, which is the enrichment of a water body with nutrients, leading to excessive algal growth and deterioration of water quality. It takes into account factors such as nutrient concentrations, algal biomass and dissolved oxygen levels. TRIX describes trophic status of the marine environment having the values between 0-10. It was presented scope of the OECD Programs on Eutrophication and applied firstly in North-west Adriatic. TRIX was adopted by The Program for the Assessment and Control of Marine Pollution in the Mediterranean for trophic classification of the coastal waters in Mediterranean Sea and by this way, the parameters of TRIX were agreed to be monitored by United Nations Environmental Program/ Mediterranean Action Plan, Water Frame Directive of EU, and was also used in various areas of the Mediterranean Sea.

TRIX have been applied since 2007 in Türkiye, to assess the eutrophication process and consequences impacts of cage fish farms in coastal marine environments. Every fish farms that located in marine areas must be analyzed the TRIX parameters, calculated the index and reported to Ministry of Environment, Urbanization and Climate Change. If the TRIX level complied to regulations rules the farm's activity will be ongoing, but not complied or find out critical levels, farm site have to change to more safe location in points of eutrophication. TRIX was founded on 4 parameters, two of them cause (nutrients TP and TIN) and other two as effect/result parameters (Chl-a, absolute percent dissolved oxygen saturation deficit). The sampling, measurements and analyses are made in May in farm sites and reported annually, for detailed application of regulation may be referred to MoEU (2020). The index can be calculated following formula, TRIX values classified as in Table 8.

TRIX = (Log10 [Chl-a * aD%O *TİN *TP] − k)/m where

Chl-a: chlorophyll-*a* concentration (μg/L),

aD%O: dissolved oxygen as absolute % deviation from saturation,

TİN: total inorganic nitrogen: (as NO_3-N + NO_2-N + NH_4-N) (μg/L),

TP: total phosphorus (μg/L)

k, and m coefficients, k= 1,5 and m= 0,833 used for Turkish coastal marine areas (may be differ in different sea or coastal sections).

Table 8. TRIX index eutrophication risk classes applied in Türkiye (MoEU, 2020).

TRIX values	Eutrophication status	Evaluation/explanations
Tİ < 4*	no eutrophication risk	May be allowed to establish net cage fish farms
4 – 5*	low eutrophication risk	May be allowed for present net cage fish farms, not allowed for new one
5 - 6*	eutrophication risk	Not allowed for new one, some limitations for present net cage fish farms
> 6*	high eutrophication risk	Not allowed for new fish farming, current farms will be closed

* For Black sea applied as +1.

4. Use and Evaluation Trophic State Indices

These trophic indices are valuable tools for researchers, environmental managers and policy makers to monitor and manage the trophic status of lakes and reservoirs. By understanding the nutrient dynamics and biological productivity of these water bodies, appropriate management strategies can be implemented to maintain or restore the ecological balance and water quality.

4.1. Inland Water Management

Management implications

Eutrophication is serious problem worldwide, also in many Turkish inland waters (lakes, logons, wetlands), brackish waters, estuaries (eg. Bafa lake, Küçükçekmece lagoon) and marine areas (Marmara and Black Sea, some sensitive bay such as İskenderun, İzmir, Golden horn, mucilage event in Marmara and the strait of Çanakkale), for aquatic ecosystems several decades. Eutrophication is driven largely due to excess nutrients associated with anthropogenic (agriculture, domestic wastes, animal breeding, fish farming, industrial effluents etc.) activities. Lake restoration/rehabilitation projects aim to lift water quality of lakes to or closer to their undisturbed conditions. It is a critical necessity to quantitatively plan and assess the recovery of lakes in restoration projects. The national managers and policy makers need useful tools that can help them learn from former experience and enable them to manage to protect and proper management. Several studies have proposed for adaptive management of eutrophication, and this approach needs continuous monitoring and TS estimation.

Drinking Water

- Trophic state indices (TSIs) are valuable tools in managing drinking water sources, particularly in lakes, dam lakes and drinking water reservoirs by providing a quantitative measure of the water body's nutrient status and its potential for algal growth. For managing and evaluating baseline establishment were done by regularly measuring TSIs helps establish a baseline of the water body's trophic status. A trend analysis based on continuous monitoring and assessment is conducted. This helps track changes over time, enabling detection of trends towards eutrophication or improvement need of drinking water source. The majority of drinking water sources need water quality management which composed of (i) nutrient load reduction (ii) algal bloom prediction and control (iii) water treatment process optimization (iv) pre-treatment requirements (v) drinking water cost management (vi) regulatory compliance and reporting (vii) ecosystem Health and Conservation
- **(i) Nutrient load reduction**; high TSI values indicate high nutrient levels (particularly N, P). Management strategies can be implemented to reduce nutrient loads from agricultural runoff, wastewater discharge (if any), and other sources.
- **(ii) Algal bloom prediction and control:** High TSIs often correlate with increased risk of harmful algal blooms. Early detection allows

for timely interventions such as aeration, algaecide application, or flow modification (in dam lakes, reservoirs) to mitigate blooms.

- **(iii) Treatment process optimization** need to plan the treatment process and their levels in the case no available other drinking water source absent.
- **(iv) Pre-treatment requirements**, understanding trophic state helps in planning the necessary pre-treatment processes to ensure water quality, higher TSIs may require more advanced filtration and disinfection processes to remove organic matter and control algal toxins.
- **(v) Cost management**, efficient management of treatment processes based on TSI data can reduce operational costs by targeting treatment efforts where they are most needed.
- (**vi) Regulatory compliance and reporting** needed to meet standards, ensuring that water quality meets regulatory standards is crucial (TSE 266 Turkish drinking water standard) TSIs provide a straightforward metric for reporting and demonstrating compliance with water quality regulations. Public communication also needs clear and quantitative TSI data can be used to communicate water quality status to the public and stakeholders, promoting transparency and trust.
- (vii) **Ecosystem health and conservation**; majority of drinking water sources are also an aquatic ecosystems that support live populations, so it should be preserved biodiversity by managing trophic levels for their welfare, maintain a balanced ecosystem, preventing the dominance of a few species (blue green algae) that thrive in eutrophic conditions. It also addresses to habitat protection that water and living things are being together. This can be achieved by managing nutrient levels and preventing excessive algal growth, the habitats of aquatic organisms are protected, supporting overall ecosystem health.

4.2. TSIs and Fisheries

TSI is an essential tool for managing inland water fisheries. By understanding and categorizing the trophic state of a water body, fisheries managers can make informed decisions to optimize fish populations, ensure sustainable practices, and maintain ecological balance. Some examples are;

- TS category: Oligotrophic (TSI < 40): Low nutrient levels, clear water, and low primary productivity. Proposed management rules; typically supports cold-water fish species (trout) practices focus on preserving water quality and preventing nutrient enrichment.
- Mesotrophic (TSI 40-50): Moderate nutrient levels and productivity, supports a mix of cold-water and warm-water species. Management may include balancing fish stocking (trout, carp, wells, eel, northern pike etc.) and monitoring nutrient inputs to maintain current conditions.
- Eutrophic (TSI 50-70): High nutrient levels, increased algal growth, and reduced water clarity. Usually supports warm-water species (carps, wells, catfish etc.), management focuses on controlling nutrient inputs, possibly through regulations on agricultural runoff and wastewater treatment.
- Hypereutrophic (TSI > 70): Very high nutrient levels, frequent algal blooms, and poor water quality. Challenges include hypoxia and fish kills in some season. Management strategies may involve drastic nutrient reduction measures, aeration, and sometimes biomanipulation to control algal blooms.
- Lake Erie, USA, has experienced significant eutrophication due to agricultural runoff and urbanization. It assessed TSI values has been critical in identifying nutrient hotspots and periods of hypereutrophic conditions. Management actions: implementation of nutrient management plans, including restrictions on P use, wetland restoration, and promotion of best management practices (BMPs) in agriculture. Outcomes: Improved water quality and reduction in the frequency and severity of harmful algal blooms (HABs).
- Lake Victoria, East Africa, the largest tropical lake, supports a significant inland fishery, including the economically important Nile perch. High TSI values have indicated eutrophic to hypereutrophic conditions, largely due to nutrient runoff from agricultural lands and urban areas. Efforts were made to reduce nutrient inputs through improved agricultural practices, sewage treatment, and community

education on environmental conservation. Outcome: gradual improvements in water quality, although challenges remain due to the lake's large size and extensive catchment area.

- Loch Leven, Scotland, shallow, eutrophic freshwater lake used for trout fishing. Monitoring TSI values has revealed trends in nutrient levels and water quality, informing management decisions. Implementation of nutrient reduction programs, including improved agricultural practices and sewage treatment upgrades. Regular monitoring of algal blooms and water quality parameters. Outcome: Stabilization of TSI values and improved water quality, benefiting both the fishery and overall ecosystem health.

In Conclusion, using TSI values and trophic categories provides a scientific basis for managing inland water fisheries. By monitoring and managing nutrient levels, fisheries managers can enhance water quality, support sustainable fish populations, and protect aquatic ecosystems. Case studies from various regions demonstrate the effectiveness of TSI-based management in addressing eutrophication and maintaining healthy fisheries.

4.3. TSIs and Aquaculture: Net cage Fish farms

Trophic Indices can be used to evaluate the aquaculture's impacts on lake ecology. For evaluation following points are checked:

- Monitoring Nutrient Inputs: Aquaculture activities often introduce additional nutrients into the water system, primarily from fish feed and waste. Regular monitoring of TSI parameters (such as TN, TP, Chl-a,) can help track changes in nutrient levels over time.
- Detecting Eutrophication: Elevated TSI values can indicate eutrophication, which can result from excessive nutrient inputs from aquaculture. This can lead to algal blooms, decreased water quality, and hypoxia, affecting the overall health of the lake ecosystem.
- Assessing Water Quality Trends: By calculating TSI regularly, changes in water quality can be identified. Increasing TSI values over time may suggest that aquaculture practices are contributing to deteriorating water quality, prompting the need for management actions.

- Biodiversity Impacts: High TSI values and eutrophic conditions can lead to loss of biodiversity. Monitoring TSI helps in evaluating how aquaculture affects species composition and abundance in the lake.
- Baseline assessment: Before establishing fish farms, we need to conduct a baseline assessment of the lake's trophic state using TSI. This provides a reference point for future comparisons.
- Ongoing monitoring: Implement a regular monitoring program to measure TSI indicators. Compare these values to the baseline to detect any changes in the trophic state. This can involve monthly or seasonal sampling to capture temporal variations.
- Thresholds and action plans: Establishing the thresholds for acceptable TSI values based on ecological and regulatory standards. If TSI values exceed these thresholds, implement action plans such as reducing nutrient inputs, improving waste management, or altering feeding practices in aquaculture can be applied.

TSI in Certification applications and audits

Aquaculture certifications (such as Global G.A.P. Aqua, ASC, BAP) mainly target to protect water environment, fish welfare and ask to the fish farms to comply related standards**.** And certification bodies monitor and audit the farms for certification needs that explained in certification schemes. In certification issues following topics are emerge relating to TS and its changing in spatial-temporal scale;

- Compliance with Standards: Many aquaculture certification programs require compliance with water quality standards. Regular TSI monitoring can demonstrate compliance with these standards, showing that aquaculture practices do not adversely affect the lake's trophic state.
- Documentation and Reporting: Maintain detailed records of TSI measurements and related water quality data. This documentation is crucial during certification audits to provide evidence of responsible environmental management practices**.**
- Corrective actions: Use TSI data to identify areas needing improvement. Certification bodies may require evidence of

corrective actions taken to address any identified water quality issues. This can involve adjusting feeding rates, enhancing waste treatment, or implementing buffer zones to reduce nutrient runoff (in land-based farms).

- Stakeholder engagement: Sharing water quality reports (including TSI data) with stakeholders, including local communities, regulatory agencies, and certification bodies are required by certification schemes. Transparent communication builds trust and demonstrates a commitment to sustainable aquaculture practices.

Practical Implementation for certification and audits

- Sampling and analysis: water samples are collected regularly from multiple locations around the fish farm site. Analyze are made for TN, TP, Chl-a and SD.
- Data Management and reporting: Using TSI, TLI, or TrTLI formulas (in Türkiye) the scores are calculated and results analyzed. Every farm should their own TSI values and track them and changes over time (trends, problems, corrective action taken, external factors that impact of TSIs etc. report to certification body and MoAF agencies.
- Audits: During certification audits, present TSI data and demonstrate how it is used to manage and mitigate the environmental impact of fish farming operations.

Application in Turkish aquaculture

Many Turkish inland water bodies are valuable sites for net cage fish farms. So this present source availability, so many dam lakes and some reservoirs are used to produce rainbow trout (*Oncorhyncus mykiss*) (Fig. 2) To establish the cage fish farm in dam lakes regulated and controlled by Ministry of Agriculture and Forestry (MoAF) general directory of Fisheries and Aquaculture (GDFA) following to Fisheries Act Nr. 1380 and related Aquaculture directive of Türkiye. However, the establishment and sustainability of fish farming need to protect water bodies which used for net cage culture. Because not all the lakes and dam lakes are suitable for fish farming, and if suitable stocking density and area use have importance, farm site selections are crucial issues for preventing water pollution and the sources' ecological integrity and production sustainability. Using TSIs to manage net cage aquaculture in lakes, dam lakes and reservoirs involves monitoring and regulating nutrient inputs and maintaining water quality to prevent eutrophication. For proper management of aquaculture such aquatic ecosystem needs following activities and management application in regard to TS and TSIs/TLIs/TrTLI.

1. Assessment and monitoring

- Baseline Establishment: Regularly measurement of parameter and estimate TSIs needed to establish baseline nutrient levels and TS of the water body before allowing net cage farms.
- Continuous monitoring: Conduction of ongoing monitoring are necessary to track changes in the TS, particularly around the net cages, to detect early signs of eutrophication.

2. Regulating nutrient inputs to the lake

- Feed Management: Since overfeeding and using high-P contained feeds can increase nutrient loads, leading to higher TSIs and potential eutrophication, it should be optimized feed types and quantities (lower feeding rates) to minimize nutrient waste.
- Feed Conversion Efficiency: For rentability and environmental protection and Improve feed conversion ratios (FCR) to ensure more nutrients are absorbed by the fish and less are released into the water.

3. Farm site selection and stocking density

- Appropriate site selection: Choosing suitable locations with good water circulation to disperse waste and nutrients, reducing localized nutrient accumulation and high TSI values, are crucial.
- Stocking density control: Maintaining appropriate stocking densities to balance fish production with the water body's ability to assimilate and process waste without significant increases in TSIs is needed.

4. Waste Management

- Waste Removal: The implement measures can ensure to remove uneaten feed and fish waste from the net cages, preventing them from contributing to nutrient loads and raising TSIs levels.
- Sediment management: Periodically monitor and manage sediment accumulation beneath cages, as nutrient-rich sediments can contribute to internal nutrient cycling and elevated TSIs, are useful activities.

5. Water Quality Management

- Water exchange and aeration: Farms need to enhance water circulation and aeration in and around the net cages to reduce nutrient concentrations and support aerobic decomposition processes, helping to keep TSIs low.
- Phytoplankton Management: Monitoring and manage phytoplankton levels, as excessive growth can indicate rising nutrient levels and higher TSIs.

6. Adaptive Management Needs

- Adjusting operations based on TSI trends: TSI data to adapt aquaculture practices can be used. If TSIs indicate rising nutrient levels, consider reducing feed input, stocking density, or increasing waste management efforts.
- Seasonal adjustments: Modifying operations based on seasonal variations in water temperature, stratification, and nutrient dynamics, as these factors can influence TSI and eutrophication risk, contribute to solve the problems.

5. Conclusions and Remarks

- TSI, TLI, OECD categories, TrTLI and TRIX are useful trophic state estimation methods. The use and effectiveness of the methods depends on water body characteristic, limiting nutrients, country, the use of the water body, the purpose etc. TRIX is devoted especially in marine areas.

- In Turkey there are several studies that published on trophic states of lake, used usually TSI of Carlson, than come TLI and OECD. The official method declared by official authorities (TrTLI) was used as minor sense. Either methods is chosen to apply, it is very important to check the correlation between the parameters (TP, TN, SD, Chl-a) and the every characteristic of the water body should be taken into consideration in final decision.
- Besides several studies were made on TSIs the managerial application and outcomes are absent in Turkey. Some official efforts were shown for application of TrTLI but not experienced and outcome yet. The TRIX has been applicated and positive outcomes were taken to protect marine areas of eutrophication's impact.
- It's need to understanding the scientific base of trophic level estimation and relating methods, sampling considerations, more accurate and economically cost effective and available methods for TP, TN and Chl-a.
- As they are important tools to establish present status of management inland water bodies, to use fisheries and aquaculture management, certifications needs the attention will be focused on the trophy indices and their application in future.

6. References

Abell, J. M., van Dam-Bates, P., Özkundakci, D., D. P. Hamilton, (2020). Reference and current Trophic Level Index of New Zealand lakes: benchmarks to inform lake management and assessment. *NZ J. of Mar.& Freshwater Res.* doi.org/10.1080/00288330.2020.1726974

Adamovich, B. V., Zhukova, T. V., Mikheeva, T. M., Kovalevskaya, R. Z., & Luk'yanova, E. V. (2016). *Long-term variations of the trophic state index in the Narochanskie Lakes and its relation with the major hydroecological parameters. Water Resources, 43*, 809-817.

Akkoz C, Yilmaz B, Kalipci E. Trophic level determination of Acı lake, lake Meke and Sugla lake. *World J Fish Mar Sci.* 2009;1(3):243-247.

Aquaculture Steawardship Council. (2019). *Freshwater Trout Standard Version 1.2.* https://www.asc-aqua.org/wp-content/uploads/2019/09/ASC-Freshwater-Trout-Standard_v1.2_Final.pdf

Aslantürk, A.& Çetinkaya, O. (2022) *Sücüllü Baraj Gölü'nün (Isparta) Trofik Durumunun Belirlenmesi*. *Acta Aquatica Turcica*, *18*(1), 1-12.

Aydın, G. B., Gürevin, C., & Öterler, B. (2022). The determination of trophic level of Küçükçekmece Lagoon Lake (İstanbul, Türkiye) by using some environmental variables with indicator benthic Macroinvertebrates findings. Acta Aquatica Turcica, 18(4), 475-485. https://doi.org/10.22392/actaquatr.1113769

Bilgin, A., 2020, Trophic state and limiting nutrient evaluations using trophic state/level index methods: a case study of Borçka Dam Lake. *Environ Monit Assess* **192**, 794 (2020). https://doi.org/10.1007/s10661-020-08741-0

Bulut, C., & Kubilay, A. (2018). *Eğirdir Gölü Su Kalitesinin Trofik Durum İndeksleriyle Belirlenmesi*. SDÜ Eğirdir Su Ürünleri Fakültesi Dergisi, 14(4), 324-338. https://doi.org/10.22392/egirdir.415073

Burns NM, Bryers G, Bowman E (2000) Protocol for monitoring trophic levels of New Zealand lakes and reservoirs. Ministry for the Environment, Wellington. 122 p.

Carlson R.E., 2007, Estimating Trophic State, *Lakeline* Spring 2007, 27: https://www.researchgate.net/publication/263042353_Estimating_Trop hic_State?enrichId=rgreq-56ddd881e682644e3e5fcf22d28dd1e3-XXX&enrichSource=Y292ZXJQYWdlOzI2MzA0MjM1MztBUzo4ODE2ODc5MzYzNzI3MzlAMTU4NzIyMjE5MzQyNw%3D%3D&el=1_x_2&_esc=publicationCoverPdf

Carlson R.E., Simpson J. (1996) A Coordinator's Guide to Volunteer Lake Monitoring Methods. *North American Lake Management Society*. 96: 305

Carlson, R. E. (1977). A trophic state index for lakes, *Limnology and oceanography, 22(2)*, 361-369. DOI 10.4319/lo.1977.22.2.0361

Dillon, P. J., & Rigler, F. (1974). The phosphorus-chlorophyll relationship in lakes 1, 2. *Limnology and Oceanography, 19(5)*, 767-773.

GLOBALGAP-AQUA. (2020). *GLOBAL GAP General Regulations*. https://GLOBALGAP-AQUAsolutions.org/annual-report-2020/services-report-2020/

GLOBALGAP-AQUA. (2021). *Integrated Farm Assurance Aquaculture Module (IFA), Control Points and Compliance Criteria*, https://www.globalgap.org/.content/.galleries/documents/220125_GG_IFA_CPCC_AQ_V5_4-1-GFS_en.pdf

Kratzer, C. R., & Brezonik, P. L. (1981). A Carlson-type trophic state index for nitrogen in Florida lakes 1. *JAWRA Journal of the American Water Resources Association*, *17*(4), 713-715.

Kutlu, B. Serdar, O. Aydın, R. ve Danabaş, D. (2017). Uzunçayır Baraj Gölü'nün (Tunceli) Carlson İndeksine Göre Trofik Durumunun Belirlenmesi. Yunus Araştırma Bülteni (1): 83-92.

MoAF (2012) Surface water quality regulation (In Turkish) Tr OG. 30.11.2012 Nr. 28483

MoEU,(2020), Regulation on environmental management of fish farms operating in the sea (in Turkish),Tr OG. 28/10/2020 Nr. 31288

NIWA (2021). Water quality state and trends in New Zealand lakes. Analysis of national lakes data ending in 2020. Prepared for Ministry for the Environment.

Nojavan A F, Kreakie BJ, Hollister JW, Qian SS. (2019) Rethinking the lake trophic state index. PeerJ. 2019 Nov 12;7:e7936. doi: 10.7717/peerj.7936

OECD, 1982. Eutrophication of Waters. Monitoring, Assessment and Control. Paris 154 pp.

Shallenberg M, van der Zon K (2021). Review of the Lake Trophic Level Index. Prepared for the Regional Council Lakes Focus Group.

Tepe, R. Karakaya, G., Şahin, A. G., Sesli, A., Küçükyilmaz, M., & Aksağan, A. (2018). Karkamış baraj gölü trofik durumu. *Int.J. of Innovative Engineering Applications*, *2*(1), 1-3.

Ülker, D., Bayırhan, İ., & Burak, S. (2020). Assessment and Comparison of Commonly Used Eutrophication Indexes. Turkish Journal of Water Science and Management, 4(1), 4-30. https://doi.org/10.31807/tjwsm.583530

USEPA, 1999. Nutrient Criteria Technical Guidance Manual: Lakes and Reservoirs, U.S. Environmental Protection Agency, EPA 822-D-99-001. 193p.

CHAPTER 6

POULTRY NUTRITION FOR HALAL MEAT

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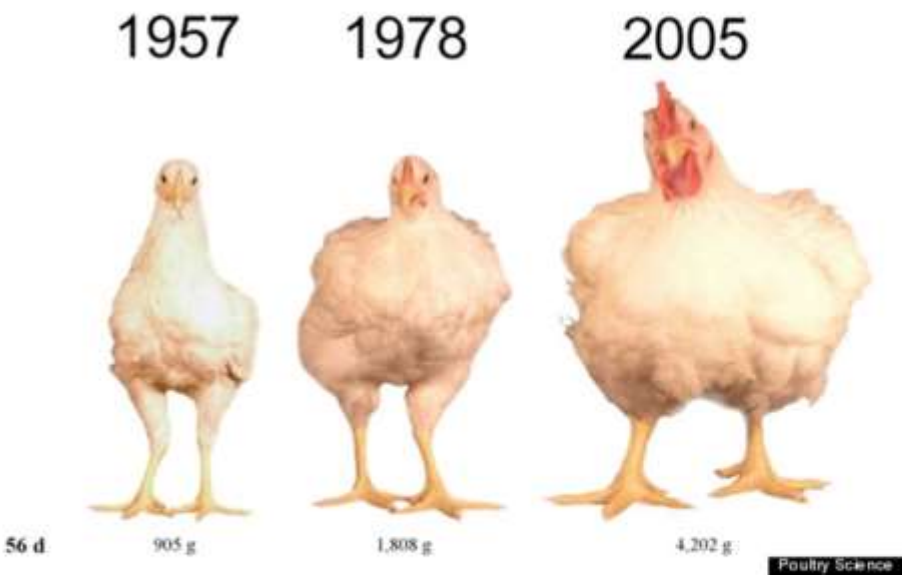
INTRODUCTION

The Muslims are introduced the term halal from birth to death. Some production sector has not considered the Halal issue for consumer. Unevitably, it is vital to recognize the Halal foods with spiritual aspects (Shafii and Khadijah, 2012). For Muslims, it is important to monitor foods which is Halal or Haram (prohibited), according to the orders by Allah. People consume poultry meat as white meat to maintain their protein requirements for growth and physiological purposes. Poultry meat is produced in poultry farm as a result of broiler production procedures. Animal feeds comprises 60-70 % of all economic inputs of poultry farm. For poultry production, corn, soybean, and its meal are main feedstuffs in chicken diets. Sometime, fish meal will be ingredient for protein source. However, because of prevailing problems and so-called "economic considerations" the poultry producers fed their animals strange and abnormal feed components. The importance of halal in animal feeding and feed manufacturing has not received enough attention, despite the vast number of research conducted to test new ingredients or additions.

In order for poultry meat to be halal, the animal itself must first be classified as halal. As poultry; chicken, rooster, duck, turkey, geese, quail, pigeon and some hunted birds are those that are considered halal. The most commonly consumed meat in white meat production is mostly chicken. For broiler farming, hybrids birds produced from Parent Stocks are used. Their body weight gain is quite fast. These birds can be slaugthered for consumption when they reached to 35-d old. These broiler chickens have been developed as a result of special and long studies, and they have the ability to convert feed to body mass in a short time and turn the feed they eat into meat. For this reason, it can be thought that the feed given to the chicken may contain hormones and is produced from non-halal ingredients. Therefore, this chapter will examine some realities in poultry production with halal concept.

HOW TO PRODUCE POULTRY MEAT?

Poultry meat, also known as chicken meat, which is one of the animal foods that is an indispensable element of adequate and balanced nutrition, becomes the focus of criticism of the media from time to time, directly or indirectly. Chicken meat is often referred to as a risky and hormone-containing food and not produced in halal way that is not suitable for consumption in Muslim world, often without consulting the opinions of experts on the subject, and with the wrong statements of people who are not experts on the subject. This misperception is accepted by the masses. Such unfortunate statements aim to prevent both our people from eating healthy with animal foods and the poultry industry, which is the most developed sector of our country's animal husbandry. These statements also will reduce consumers' protein intake from chicken. This will cause their health negatively. While this is the case, prejudices about chicken meat, one of the sources of white meat, may cause our people not to have an adequate and balanced diet. In addition, our poultry sector is exposed to huge economic speculations and life-threatening risks.



Picture 1. Historical development of broiler chicken at 56-d old (Zuidhof et al., 2014).

Contrary to popular belief, broiler chickens used in poultry farming in our country are not genetically modified animals or injected with special drugs. They are animals whose growth rate has been increased as a result of breeding studies that lasted more than a century and continue today (Picture 1). Broiler chicks can reach a live weight of 2 kg or more in less than 35 days. In this rapid growth, the contributions of climate-controlled chicken coops, full-day lighting and first-class feed based on soy and corn (3200 Kcal metabolizable energy and 230 grams of crude protein per kg, adequate and balanced vitamin and mineral content) are inevitable. On the contrary, broiler chickens raised in less controlled chicken coops and in open areas grow more slowly, although they consume more feed to keep their body temperature balanced. However, since the initial ambient temperature in controlled chicken coops is gradually reduced over time from 33 ˚C to 24 ˚C, broiler chickens do not have to consume more feed to adjust their body temperature, and at least half of each gram of feed they consume is used to form body building blocks (white meat production) can be evaluated. Broiler chicken producers ensure that broiler chickens are housed according to the temperature sensitivity mentioned above, that they receive clean oxygen as much as they need, and that they are housed on quality litter (that does not cause wetness and dust) during all production stages, and they guarantee the cleaning and disinfection of the coop. In broiler chicken production, these precautions are not limited only to the growing period, but are also continued during transportation and slaughter. The number of chicks per square meter in chicken coops is more than 10. This accommodation frequency complies with EU norms. The chicks, fed with high quality feed in very comfortable chicken coops, eat and drink water 24 hours a day without spending any time or energy searching for or digging for feed, and since they sleep or remain inactive in the remaining time, they mostly use the energy and other nutrients they take in for their own bodies. Broiler chicken producers and integrated companies know very well that if animal welfare is not respected and the comfortable environments that chicks need are not provided, efficient production cannot be achieved. As a result of these special conditions and quality feeding practices, this rapid growth achieved in a short time causes different interpretations by people who are not experts on the subject, and ultimately causes consumers to misperceive the consumption of chicken meat.

There is absolutely no hormone application to ensure rapid growth of chickens. Scientific research on the use of hormones in chickens has shown that it is not possible to get the expected results from hormones and that giving hormones to chickens is not economical and feasible. Even if the hormone is free, procedures such as capturing the animal and injection of hormones will cause stress and pain in the animals, causing more live weight loss rather than live weight gain. In addition, it is obvious that, for example, in a chicken coop with a capacity of 100 thousand, the process of injecting hormones into the body of each broiler chick is quite laborious and impractical. Because the use of hormones in the poultry sector is prohibited in our country, and to date, no case of hormone use has been reported by authorized institutions.

There is no use of hormones even if chickens or broiler chickens get sick. Because disease treatment in the poultry industry is quite expensive and inconvenient, health protection measures are taken only by agricultural engineers, zootechnicians and veterinarians and flock management is implemented in the best possible way. These measures and practices begin with the supply of healthy breeding eggs and hatchery management, and continue with vaccinations given to the chicks before they are transferred to the coop. These vaccines do not have any hormone-like effects. These vaccines are the elements that provide immunity to chicks against possible disease factors.

Another source of concern for consumers relates to the use of antibiotics. The legal regulations regarding the use of antibiotics are in line with the EU legislation, and the addition of antibiotics to feed as feed additives is strictly prohibited. For this reason, many researchers have focused on scientific studies to identify alternative feed additives to antibiotics. As a result of R&D and field studies, commercial forms of plant extracts, probiotics and prebiotics that do not have any negative effects on human and animal health are also preferably used by broiler chicken breeders. The purpose of using these alternative products is to ensure that chicks benefit more from the feed they consume by increasing the immunological function and absorption function of the digestive system. The main thing in feeding broiler chickens is that the feed must be well formulated in terms of nutrients, vitamins and minerals.

Apart from hormone and antibiotic usage, the halal issue is becoming the main concern while the Muslims are more sensitive to know whether their foods are halal or not.

HALAL CRITICAL CONTROL POINTS

Halal Control Points (HCP) are marked on the procedure of halal meat production from livestocks. Halal poultry meat production can be done by obeying some rules at halal critical control points (HCCP, Figure 1, Shahdan et al., 2017).

For some decades, the governmental and nongovernmental organizations have been practised the authority to certify halal animal foods. Depending on the country and dominant schools of thought, the criteria for labeling a product as halal may differ. For example, while electrical stunning (ES) before slaughter in halal meat production is considered permissible in some countries, it is not allowed in others. Lever and Miele (2012) reported that the efforts to harmonize the global halal market through the use of standards that often include economic as well as animal welfare factors, which makes their acceptance by others difficult. Nearly 8 years ago, Shahdan et al. (2016) explained 6 control points (CP) for halal poultry slaughter based on the fundamental guidelines derived from the Qur'an and the Hadiths. Their control points are defined as following;

- CP1 is the permissibility and prohibitions with poultry feeding.
- CP2 is the Islamic requirements for humane managing of poultry.
- CP3 is that immobilization methods during slaughter are permissible.
- CP4 is the permitted equipment for cutting purpose and neck cutting.
- CP5 refers to the need to provide sufficient time between slaughter and scalding to maximize complete bleeding and also to ensure that the chickens die before entering the scalding tank.
- CP6 is the restriction imposed to prevent adulteration of meat with fecal residue and blood.

These control points are illustrated in Figure 1. These points have also critical limits (CL). The CL for each CP for halal poultry slaughter will enable the determination of acceptability limits that will prevent a product from becoming haram (forbidden) or shubhah (doubtful). For example, electrical shocking can cause mortality prior to neck cutting when its tuning is done irregularly, the extended time, depending upon the animals' body composition and size. Then, how these dead animals would be halal?

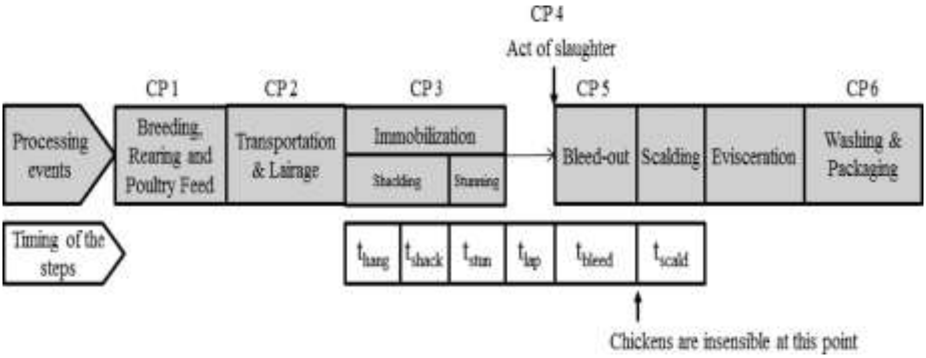


Figure 1. Steps in chicken processing plants along with the critical limits (CL) for control points (CP) for halal slaughter (Shahdan et al., 2017).

Be assured of halal slaughter in halal meat production Halal critical control points to must be monitored with appropriate system from poultry feeding to slaughtering. In order to ensure halal traceability in meat production, halal critical control points must be well known and meticulously implemented. In this context, each aspect or operation on the farm, from feeding the chickens to the final packaging stage, it is very important to determine and follow the halal critical control points (Batu, 2023).

At the same time, “Good Farming Practices” in poultry industry (such as right selection of suitable farm site, infrastructure and facility at farm, maintaining workers welfare, free access to water and feed to animal, gentle handling of animals, sanitation, recording, waste & pollution management, ontime vaccination and medication, and precaution for biosecurity, etc) will give advantage to both of the farmer and community. One of the interests was that the meat producing farm animals were safe and healty. These make closer to farmer and consumers to produce or consume Halal poultry meat in more proper manner (Ramli et al., 2020).

Table 1. Explanation of halal critical control points in poultry industry (adopted from Shadan ve ark., 2016)

Reqirements for Halal meat	**Conventional practices**	**Proposed Control Points (CP)**
Halal feed ingredients	Unkown feed ingredients Rendering materials, unhealthy substances, blood and pork components in poultry feed	CP 1 Poultry breeding, rearing, and feeding
No stress to animal	Legs problems and ascites	
Behaving humanly handle animals Obtaining feed and water to animals during transport	Crowded populations Hot condititions and insufficient ventilation Longer starvation	CP 2
Prohibition of restraining the animal after its neck has been cut	Remaining shackled after neck cutting	CP 3 Immobilization for slaughtering
Humanly handling when slaughtering the animal Prohibition of killing by means other than decapitation, such as stunning Allowing blood flow after neck cutting	Metallic shackle can be painful (legs bruises) Stunning can cause death	
Reciting tasmiyah to sacrifice for Allah Using a sharp knife No witnessing the other animals Effective cutting for blood flow Turning animal's head to the Kaaba while neck cutting	Reciting tasmiyah irregularly Unusable knife for cutting Chained chickens are slaughtered in orde Not effective and proper cutting by knife Is animal facing kaaba?	CP 4 Act of slaughter
Not allowing of killing by means other than decapitation, such as drowning in a scalding tank	Birds may not be dead because of the short time between neck cutting and boiling.	CP 5 Time for full bleed-out
Package clean meats without any haram, poisonous or harmful substances and materials	Mixing with other meats when different animal species are processed in the same facility Using some blood and pig by-products in secondary meat products	CP 6 Cleaning and packaging

The above control points must be considered altogether. If one of these is not applied, the end product will become haram.

POULTRY FEEDS

Animal feeds are divided into multiple categories, depending upon their nutritional value and intended use. Depending on the industry spectrum, various countries have named these feed types differently, but common to all of them are compound feed, energy feed, protein feed, supplement feed, premixes, concentrates and forages, medicated feed and feed additives. Amongst these the commercial concentrates (compound or mixed feed) are known as mixtures manufactured by using feed materials or feedstuffs enriched feed additives and offered to animals ad libitum or as meals. Nowadays, animal feeds are fuctioned as complete diet or as complementary feed. The word "complete" refers to the feed that is compositionally suitable for daily nutrition, and the word "complementary" refers to the feed that needs to be mixed with other feeds due to the high amount of some substances. The latter one is mainly used for ruminants rather than poultry. In the event that chickens are allowed to ranges to pasture, the latter feed materials may be used fort he supplementaion of protein or minerals.

Energy feeds are generally produced from variously refined cereal grains and other plant products such as wheat, corn, oats, barley, sorghum, potatoes, wheat bran and center, corn cob, rice bran and beet pulp. Among all these ingredients, the most commonly used ingredient in poultry farming is corn kernels due to their higher energy and carotene content.

Protein feeds contain more than 20% protein of plant or animal origin. While plant-based feedstuffs mostly consist of seed and gluten meal, animal-based feedstuffs are feeds obtained from various land animals, such as blood, bone and meat meal, and marine animals, such as fish meal. In poultry, dominantly used ingredients is full fat soya bean and its meal.

Supplementary feed is a kind of feed materials normally used along with basic feedstuffs for increasing feeding value of the offered feed. This feed is either rich in protein, energy or minerals & vitamins. In poultry, these are considered for diet formulation.

Premixes are added to diets. These include high concentration of one or more substances such as vitamins, mineral or other chemicals and are used dietarily with appropriate mixturing methods in poutry.

Concentrates often contain grains and grain by-products such as wheat bran, oilseed meal, and gluten meal or fish meal. These ingredients are essentially diluted or mixed with other feed ingredients, thus making complementary or complete feed or dietary formulations. These are used in poultry for making diet balanced with energy and protein.

Roughages are the plant-based category of feed consisting of fresh choppings, silages, pasture, and rangeland plants. However, they are not used for intensive poultry farming, except free range production methods. Silages is not used for poultry nutrition, except some scientific purposes (Allen et al., 1940; Krogdahl, 1985).

Medicated feeds a special type of feed offered to animal only in case of disease such as salmonella and coccidiosis and usually contain one or more veterinary drugs. They are used nutritionally directly or by dilution into feeds or water.

Feed additives are non-nutritional substances, including enzymes, probiotics, herbs, and antibiotics, that are deliberately added along with essential feed ingredients for the production of compounds or other types of feed for the purpose of stimulating growth, enhancing feed intake, palatability, and preservation. In poultry, prebiotics, probiotics and plant extracts are used either for supplementary or medication purposes.

In diets of poultry will include corn, soybean, sorghum, barley, Distiller's Dried Grains with Soluble (DDGs), sunflower seed meal, cotton seed meal, rape seed meal, fish meal, limestone, dicalcium phosphate (DCP), salt, amino acids, enzymes, etc.

HALAL CONCEPT IN POULTRY NUTRITION

Islam is the fastest growing religion in the world, with 57 countries worldwide and a population of 1.6 billion, accounting for 25% of the total world population. The continents Asia and Africa are the 2 regions where Muslims live the most, with population shares of 62% and 35% respectively, while approximately 10% of the world's Muslims live in Europe. It must be mentioned that many people are becoming Muslim due to recent “Gaza Massacreas” by Israil.

Eating and drinking are very sensitive issues in Islam. Althouh our planet is full with living and non-living organisms, some of these are not allowed for consumption in Islam. Islam has imposed certain number of restrictions in this regard. Halal means permissibility while Haram is known as prohibition. In the literature on Halal, the discussion usually revolves around the main prohibitions such as the consumption of pork, blood, carrion and even Halal animals not slaughtered according to Islamic requirements, intoxicants and their products. Contrary to what is expressly prohibited, among land animals, Halal (permissible) food sources such as mammals, fish or birds and their products, such as eggs, meat and milk (e.g. sheep, goats, camels, cattle, roosters, pigeons, dove, quail, sparrow and fish) are considered suitable for Muslim consumption. Muslims consume meats produced and slaughtered according to Islamic rules. For this reason, feeding animals on which they grow and live is also very important as per the Islamic Jurisprudence. Evethough some animal feeds are economical and readily available, Muslims are sensitive their usage in poultry feeding due to presence of Haraam and doubtful ingredients. One of the common practices followed worldwide both in developed and developing countries is utilizing rendering materials obtained from slaugtherhouses (dead animals and their offals, etc) in feed formulation for compensation dietary protein needs cheaply. Therefore, Halal issue was mentioned here to understand poultry meat production for soul satisfaction. Animal by-products such as meat meal, bone meal and feather meal, including animal waste from pigs, cattle and poultry, highlight the integrity of Halal and Tayyib. These problems regarding ensuring feed and food safety in Europe with respect to transmissible spongiform encephalopathies (TSE) and bovine spongiform encephalopathy (BSE) are solved without taking into account the Halal issue. The use of probiotics is also a controversial area as the microorganism can come from the Haraam environment or from the Haraam animal source. Additionally, the use of tissue culture in meat production is controversial from an Islamic perspective. These are critical issues that Islamic Law must address.

As farmer or producer, Muslims must behave humanly in caring, breeding, managing and feeding of animals in their farms or house areas without giving pain to them. They are free to live the way they like and consume and behave the way they have been natured to do so. The general rule in animal nutrition, we should offer animals the feeds which we can eat without thinking. In reality, “feed is the essence of food” because animal turns what it eats into a product. Doubtful considerations and applications regarding the halalness of animal feed may ultimately affect the halal status of halal food products, thus attracting the attention of Islamic Law.

Poultry feed is prepared from feedstuffs, ingredients, additives and supplements whether processed, semi-processed or raw, which is intended to be fed directly to poultry. Recently, there has been somen concern on the Halal integrity of the poultry feed. The safety of the poultry feed had also been discussed and attracted the attention of the society in the world. Halalan Toyyiban refers ‘lawful’ and ‘wholesome’ in Islamic context which also relates to the feed/food safety as well. The safety and cleanness issues involved include the use of some unsuitable ingredients that can trigger toxic contamination and sometimes lead to higher levels of bacteria in poultry. Beside Halal issue, we should look into microbiological contamination as well as chemical hazards which may cause food injurious to the health of the consumers (Ashraf et al., 2018).

Chickens are omnivorous animals in terms of their feeding habits. In other words, they can eat both plant and animal sources. It means that chickens consume grains, seeds or other vegetation and insects, worms or small reptiles (mice, lizards, etc.) in natural life (free-range chickens do same). At the same time, they can consume celllale substances. They mix the soil to find seeds and insects. In industrial poultry farming, producers provide chickens appropriate diet matching their metabolic and physiological needs. In this industry, some cellale ingredients (rendering materials) is contributed to broiler diet. In order to produce halal meat, the feeding of broiler chicks must be based on Islamic rules. Day old hybrid chicks is used for production. Their number is the upto poultry house and economical capacities. These chicks are placed in floor compartment cowered with wood sawdust. The mixed feeds is prepared as Table 2, depending upon the available feed ingredients but not doubtful ingredients for halal concept. These feeds or diets are called starter for 1-11 days, grower for 12-25 days, finisher 1 for 26-39 and finisher 2 for 40-42 days. The last diet finisher should be discarded if there is no cellale concept.

The other procedures will be done by obeying the halal critical control poinst as Shahdan et al. (2016, 2017) stated.

Table 2. The proposed broiler diets for Halal chicken meat production

Feed ingredients	Starter	Grower	Finisher 1	Finisher 2
Plant oil	1,20	2,20	3,20	3,30
Wheat	11,00	11,00	11,00	11,00
Yellow corn	46,00	49,00	53,50	57,00
Soya bean meal (48 % CP)	32,00	28,50	24,50	21,00
Full fat soya bean	6,00	6,00	4,60	4,40
DCP	1,70	1,32	1,29	1,50
Dl-methionine	0,20	0,18	0,18	0,15
Limestone	1,01	0,93	0,85	0,77
L-lysine	0,27	0,20	0,21	0,24
L-threonine	0,12	0,09	0,09	0,08
Salt	0,32	0,33	0,33	0,34
Vit+ Min Premix	0,25	0,25	0,25	0,25
Nutrient contents				
DM %	90	90	90	90
CP %	23	21,5	19,5	18
ME, kcal/kg	3000	3100	3200	3225
Crude ash,%	0,96	0,87	0,78	0,74
Ca,%	0,48	0,44	0,39	0,38
Na,%	0,16	0,16	0,16	0,16
Cl	0,23	0,24	0,24	0,24
Meth+Sis,%	0,95	0,89	0,83	0,76
Lysine,%	1,46	1,31	1,17	1,10
Threonine,%	0,97	0,89	0,81	0,74

Above diets were used by Çayan (2018) in his PhD thesis, broiler chicks reached 2635 g at the end of 42-d, as if the commercial target body weight.

LEGISLATION

Clean and healthy nutrition is a human right. Halal food is part of freedoms belief. Halal certification covers not only food but also cosmetics, service centers, renewable energy, tourism, agriculture and transportation etc. services and activities is included. Halal certification studies have made accreditation and standardization mandatory. Halal certification and standardization studies have begun in the world. One of them is the Standards and Metrology Institute of Islamic Countries (SMIIC). This institute has published TS OIC/SMIIC 1:2011: General Guidelines. Halal Food Standard, along with two other guidelines. This standard contains halal food criteria and procedural rules that must be followed in an Islamic perspective. To ensure the participation of academics on the subject and to open the content of SMIIC to scientific discussion (Şimşek, 2013).

Feeding animals with impure substances is an ancient practice and has long been debated by Muslim scholars, and these arguments are divided into two groups; feeding edible and inedible animals (Muflih et al., 2017). According to Hanbali and Maliki, it is permissible to feed inedible animals with non-halal feed. The opinions are based on the fact that animals such as dogs, cats and other domestic animals such as eagles are not intended for consumption, so feeding them is not haram due to the scarcity of halal food for Muslims (Ramli et al., 2018). On the other hand, Muslim scholars express different opinions about feeding non-halal feed to edible animals. According to the Hanbali sect, if meat and milk are not consumed in a short time, it is permissible to feed impurity and filth to animals (Abdul Cader, 2015). Brunei's fatwa also discourages the feeding of meat and dairy animals with contaminated feed and the sale of these animal products without proper quarantine processes (Pauzi and Man, 2015). The animal is allowed to be consumed by Muslims after being kept away from dirty feed for about 40 days.

Cellala refers to the class of animals that are repeatedly fed with dirt and foreign substances or regularly fed with dung, causing the aroma, taste and color of the meat to change (Jamaludin et al., 2014). On the other hand, it should not be forgotten that an animal can only be believed to be jalala if most of the feed it eats is impure and there are visible changes in the physical properties of the meat and milk. This is because a few animals normally swallow a certain amount of droppings or their own feces (coprophagia), and this does not change the physico-chemical properties of meat and milk. Unless the animal returns to normal production through quarantine and is fed clean feed before slaughter, cleaning or cooking methods do not eliminate the legal makruh provision (Rahman and Laziz, 2012).

The fashion of feeding meat animals with animal waste and animal by-products is a widespread custom in rural areas and has also been noticed in many Asian countries. This has initiated an issue in accordance with Islamic law and created doubts among the Muslim community due to the lack of clear answers and solutions. There has been debate between the halal animal feed scenario and the application of animal by-products and their extracts to the animal diet, including pigs (Norhana et al., 2012). Some scholars think that offering food containing any part of a haram animal is impure and slaughtering it is haram. Some other scientists believe that an animal that lives in filth and constantly eats foreign substances is classified as infected and is banned. “cellala” is a term used to describe animals that always or almost never eat the waste of other animals or pigs and their derivatives, resulting in changes in their aroma and taste. Our Prophet prohibited eating the meat and milk of such animals. However, the term "cellala" will not be sufficient for animals fed with soil and clean feed. Most Muslims believe that the feed for an animal should be a plant-based diet, whether raised for meat, milk or eggs. Some other Muslims accept it as true that only pork and non-halal animal by-products are prohibited in animal nutrition. For this reason, Saudi Arabia banned the sale and export of animals fed a diet containing by-products of illegal animals (Shaikh and Sharma, 2015).

Enzymes are used in baking, fruit and vegetable processing, cheese making, and beverage and food production. Enzymes can be obtained from animals, plants and microorganisms. They can be halal or haram depending on the source. If the source is Halal, it is considered Halal, otherwise the enzyme will be questionable. If enzymes are obtained from non-halal animals or feed environments, the inclusion of these enzymes in poultry feed may affect the halal status of the feed. Pepsin and catalase are extracted from bovine/pig stomach and bovine liver, respectively (Bawa and Anilakumar, 2013). Some enzymes are obtained directly from plants or animals through fermentation, while others are biologically engineered. Synthetic enzymes are halal only if the processing and extraction are produced by halal means. Enzymes obtained from an animal source are preferably not used for feed from a halal perspective (Ermiş, 2017).

Feed is the most important input in chicken meat production. In addition, in many farms, it is quite common to add a "protein supplement" to animal feed. These “protein supplements” can be produced from slaughterhouse byproducts and other ingredients. Ruminant byproducts are not used but some farms are used poultry rendering products. However, chicken meat produced by feding of this type of feed ingredients is not considered as halal way of feeding. Because the dead animal things like blood, bones, and chicken droppings. It is forbidden for consumption by Muslims. These feed ingredients are equivalent to cellal (Çayıroğlu, 2014). Cellale is described as an animal addicted to eating dirt. According to the Islamic sources, it is haram to eat the meat of such animals (Çayıroğlu, 2014).Therefore, feed made from animal carcasses should not be used in Halal poultry meat production.

Muslim individuals pay attention to the halal nature of the foods they eat and drink. Halal production requirements are formulated into rules and procedures in the diet, in terms of halalness, taking into account what is allowed and what is prohibited. This study was prepared with the aim of determining some halal critical control points that may be important in enlightening producers and consumers and revealing the basic principles of halal chicken production. Commercially processed chickens are raised on specially prepared poultry farms. The used feed for halal poultry should also not contain any animal by-products or other dead animal by-products, which is a common practice in European countries. To produce halal chicken meat, chickens must be fed with halal and clean feed. Before the slaughtering process, if possible, electrical stunning should not be applied and the animal should be slaughtered with a sharp knife without causing any pain. The traditional method of slaughter in Islam is to cut off the lower jaw, jugular veins, trachea and esophagus without cutting off the head. The slaughtering of chickens should be cut by an adult Muslim person of sound mind, mentioning the name of Allah. It is preferred that the sacrificing must be done properly by hand. In halal poultry meat production, post-slaughter feather plucking should be done using dry or steam plucking techniques, if possible. For feather plucking, it is sufficient for the boiling water in which the chicken will be soaked to be at most 52^{o}C and for its body temperature to reach 42^{o}C. Thus, since the feathers will be plucked easily and hygienically, the chicken meat will be healthy in terms of food safety. For halal chicken meat production, previously determined critical control points must be regularly inspected by halal food experts. For this reason, halal meat producers should always employ a sufficient number of halal food experts to ensure that halal control can be effectively implemented (Batu, 2023).

Finally, it can be seen our wholly Quran that Allah SWT said:

“He has only forbidden (haram) to you dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah SWT. But whoever is forced [by necessity], neither desiring [it] nor transgressing [its limit], there is no sin upon him. Indeed, Allah SWT is Forgiving and Merciful" (al-Baqarah, 173).

"He has only forbidden to you dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah. But whoever is forced [bynecessity], neither desiring [it] nor transgressing [its limit] - then indeed, Allah SWT is Forgiving and Merciful" (an-Nahl, 115).

CONCLUSION

The Muslims consider the Halal status of foods they eat. For this reason, some Islamic Countries have been constructed the legislations on the consumption of al-jallalah and the non-halal feeding practices. There have been some solutions for labelling halal foods and animal feeding procedures. Proper quarantine process should be applied to remove infectivity from jallalah animal to pledge the halalan toyyiban feature of the animal-based products. In animal feed industry and farms, the Halal Critical Control Points must be assessed from farm to fork, by considering animals, their feedstuffs with how to feed them, to serve the customers halal animal foods. It further recommends that the Halal certification authority should consider to include certification of poultry feed under its Halal certification scheme. To protect the benefit of the Muslim consumers, a Halal standard for Animal Feed Production should be developed. Hereafter, animal farmers have a choice the feed to produce Halal meat for Muslims. Finally, we want to focus on that if someone is not saying "Bismillahi Allahu Ekber" before using knife, the consumers should know that the other halal procedures will be lost.

REFERENCES

Abdul Cader, A. (2015). Islamic challenges to advertising: a Saudi Arabian perspective. *Journal of Islamic Marketing*, *6*(2), 166-187.

Allen, P. W., Jacob, M., Sjolander, N. O., McBee, J. A. (1940). Legume silage as a poultry feed. https://trace.tennessee.edu, Reaching time: 30.06.2024.

Ashraf, A., Abd Rahman, F., Abdullah, N. (2018). Poultry feed in Malaysia: an insight into the Halalan Toyyiban issues. In *Proceedings of the 3rd International Halal Conference (INHAC 2016)* (pp. 511-531). Springer Singapore.

Batu, A. (2023). Tavuk eti üretiminde helal kritik kontrol noktalari. *Helal ve Etik Araştırmalar Dergisi*, 5(1), 23-36.

Bawa, A. S., Anilakumar, K. R. (2013). Genetically modified foods: safety, risks and public concerns-a review. *Journal of Food Sience and Technology*, 50(6), 1035-1046.

Çayan, H. (2018). Vejetaryen beslemenin tavuklarda verim performansi, ürün kalitesi ve organoleptik özellikleri üzerine etkileri. Kırşehir Ahi Evran Üniversitesi, Fen Bilimleri Enstitüsü Zootekni Anabilim Dalı, Doktora Tezi.

Çayıroğlu, Y. (2014). İslam hukukuna göre Helal gıda. Çağlayan Matbaası, 454 sf, İstanbul.

Ermiş, E. (2017). Halal status of enzymes used in food industry. *Trends in Food Science & Technology*, *64*, 69-73.

Jamaludin, M. A., Ramli, M. A., & Rahman, S. A. (2014). Al-Jallalah: konsep dan fatwa semasa di Malaysia. *Journal of Fatwa Management and Research*, *3*(1), 31-44.

Krogdahl, Å. (1985). Fish Viscera Silage as a Protein Source for Poultry: I. Experiments with Layer-type Chicks and Hens. Acta Agriculturae Scandinavica 35(1): 3-23.

Lever, J., Miele, M. (2012). The growth of halal meat markets in Europe: An exploration of the supply side theory of religion. *Journal of Rural Studies*, *28*(4), 528-537.

Muflih, B. K., Ahmad, N. S., Jamaludin, M. A., Nordin, N. F. H. (2017). The concept and component of contaminated animals (Al-Jallalah Animals). *International Food Research Journal*, *24*.

Norhana, M. W., Dykes, G. A., Padilah, B., Hazizi, A. A., Masazurah, A. R. (2012). Determination of quarantine period in African catfish (Clarias gariepinus) fed with pig (Sus sp.) offal to assure compliance with halal standards. *Food chemistry*, 135(3), 1268-1272.

Pauzi, N., Man, S. (2015). Haiwan jallalah dari perspektif Islam: analisis fatwa Malaysia dan negara Brunei Darussalam. *Jurnal Fiqh*, *12*, 57-78.

Rahman, M. N. A., Laziz, F. D. M. (2012). The importance of quarantine period for the jallalah animals in determining the status of halalan thoyyiban of the meats. *Buletin SSMP*, *5*.

Ramli, M. A., Nuba, M. S., Rosele, M. I., Abdullah, A. B. (2017). Perspektif Ibn Taimiyyah Tentang Al-Tasyabbuh Dalam Sambutan Perayaan Bukan Islam (Ibn Taimiyyah Perspective about Al-Tasyabbuh in the Celebration of Non Muslim Festival). *UMRAN-Journal of Islamic and Civilizational Studies*, *4*(1).

Ramli, M. H., Rosman, A. S., Sikin, A. M., Jamaludin, M. A., AjmainJima'ain, M. T. (2020). Halal Assurance at Farm Level in the Poultry Supply Chain. *Journal of Islamic, Social, Economics and Development*, 5(31), 1-11.

Shafii, Z., Khadijah, W. M. N. W. S. (2012). Halal traceability framework for halal food production. *World Applied Sciences Journal*, *17*(12), 1-5.

Shahdan, I. A., Mac Regenstein, J., Rahman, M. T. (2017). Critical limits for the control points for halal poultry slaughter. *Poultry Science*, *96*(6), 1970-1981.

Shahdan, I. A., Regenstein, J. M., Shahabuddin, A. S. M., Rahman, M. T. (2016). Developing control points for halal slaughtering of poultry. *Poultry science*, *95*(7), 1680-1692.

Shaikh, F., Sharma, D. (2015). Islam and consumption: Religion interpretations and changing consumerism. In *Islamic Perspectives on Marketing and Consumer Behavior: Planning, Implementation, and Control* (pp. 103-128). IGI Global.

Şimşek, M. (2013). Helal belgelendirme ve SMIIC standardı. *Journal of Islamic Law Studies* (22):19-44.

Zuidhof, M. J., Schneider, B. L., Carney, V. L., Korver, D. R., & Robinson, F. E. (2014). Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. *Poultry science*, *93*(12), 2970-2982.

CHAPTER 7

USE OF PLANT TISSUE CULTURES IN SALT STRESS STUDIES

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1. INTRODUCTION

The fact that irrigable agricultural areas in the world are faced with the increasing salinity problem negatively affects agricultural food production. Meeting people's basic food needs depends on increasing agricultural production by making the best use of agricultural lands. Therefore, reducing or eliminating economic losses in agricultural saline soils requires selecting the most suitable plant species that can be grown in these soils (Şen A. 2005).

1.1 Salinity

Salinity is one of the important factors that negatively affects productivity in agricultural areas. In our country, it is 2-2.5 miles in total. A salinity problem is observed in an area of 1000 ha (Munsuz et al. 2001). The salinity problem naturally occurs in arid and semi-arid regions and in areas with insufficient rainfall. In areas opened to irrigation, it occurs when salts in the ground water rise to the upper layers due to excessive irrigation. Plants react differently to salt concentrations. Some plants may be more salt tolerant. In addition, the response of plants to salt varies according to their development status, and it is known that plant families and even varieties within a species react differently to salinity. The period of germination and seedling development in saline conditions is the most critical period in the total life cycle of the plant (Katerji et al. 1994; Wang and Shannon 1999; Almansouri et al. 2001).

Today, plant growth and development are increasingly limited due to environmental stresses. One of these important stress conditions affecting plant growth and development is salinity. “Salinity” describes the presence of different salts in soil or water at concentrations that may inhibit plant growth. These salts are generally chlorides (NaCl, CaCl2, MgCl), sulfates (Na2SO4, MgSO4), nitrates (Na2NO3, KNO3), carbonates and bicarbonates (Na2CO3, NaHCO3) and borates. However, the most common salt form in nature is NaCl (Küçükahmetler, 2003). Salts in the soil prevent water from being absorbed by the seeds by increasing the osmotic pressure or negatively affect germination due to the toxic effect of Na+ and Cl- ions (Kaya et al. 2005, Okçu et al. 2005; Sadeghian and Yavari, 2004). Some of the studies carried out to determine the salt tolerance of plants are field trials and some are seedling trials. Another application used to determine salt tolerance is seed germination tests. In addition, studies continue to reveal differences at the variety level in different plant species using tissue culture methods. Thus, it is possible to work under fully controlled conditions by eliminating all kinds of environmental factors and differences that may arise from nutrition (Yaşar, 2003). With this method, research has been carried out on economically important species such as tomato (Emilio A. Cano et al., 1998), rice (L. Khaleda et al., 2007), and wheat (Akhtar N., 2006).

1.2 Tissue Culture and Techniques

Plant tissue culture is the process of cultivating new tissues, plants, or plant products in a controlled environment using plant parts (explants) such as cells, tissues, or organs. This is done in an artificial nutrient media under sterile circumstances.

The purposes of plant tissue culture can be briefly listed as follows;

- Developing new varieties and creating genetic variability in existing varieties
- Protection of endangered species
- Production of species that are difficult to reproduce.

Some application areas of plant tissue cultures in plant breeding; It can be said as embryo culture, somaclonal variation, *in vitro* selection, gene transfer and micropropagation after interspecies hybridization (Babaoğlu, M. 2002).

1.2.1 Organogenesis: It is the process that puts pressure on cells and tissues and causes some changes, leading to the formation of a unipolar structure called shoot or root draft, whose vascular system is connected to the tissue from which it originates.

1.2.2 Somatic embryogenesis: It is a process that leads to the formation of a bipolar structure with an independent vascular system and containing the root and shoot axes. Embryos that develop from vegetative cells are also called somatic embryos. Because somatic embryogenesis develops from the cells of individual plants, plants derived from somatic embryos form genetic clones. In addition, just like the embryo that develops from the fertilized egg, somatic embryos in dicotyledonous plants also go through the "globular", heart, torpedo and cotyledon formation stages, and they also have a stem-root axis and can be easily separated from the tissue because they do not have vascular connections with the actual tissue.

We can say that the factors affecting somatic embryogenesis are explant source, genotype, content of the nutrient medium, and environmental conditions (Babaoğlu, M. 2002).

1.2.3 Micropropagation: Plant propagation involves the cultivation of new plants by utilizing various plant parts such as embryos, seeds, stems, shoots, roots, and calluses. These plant parts are placed in a controlled environment with artificial nutritional media, ensuring a sterile condition, in order to facilitate the development of a fully formed plant. The significance and benefits of micropropagation in the context of plant breeding and genetics; Acquiring plant material that is devoid of illnesses and pests In mass production, the plants produced exhibit both morphological and genotypic similarities. This method also allows for a shorter culture time compared to conventional methods. Additionally, it facilitates the creation of species that are difficult to breed. Moreover, it enables the rapid production of selected particular or better genotypes. Lastly, it requires less rootstock for production. We can categorize it as the acquisition of novel varieties/genotypes resulting from somaclonal variation.

1.2.4 Somaclonal variation: In plant breeding, variation that is considered advantageous in cases where natural variation is narrowed or it is difficult to create variation is seen as a new source, and all of these hereditary changes occurring in tissue culture are defined as somaclonal variation. The origin of somaclonal variation is the period of culture stimulation, the period of development of the culture, and the period of plant regeneration.

Somaclonal variation has advantages and disadvantages, and we can list them as follows.

Advantages;

- Convenient as a rapid source of variation.
- Some changes may occur at high frequencies.
- Agronomic characteristics may vary.
- New variants may emerge.
- New varieties can be produced.

Disadvantages;

- Somaclonal variation causes large losses in microproduction.
- Plant breeding techniques in plant biotechnology are based on regeneration from somatic cells. Such plants are under the influence of somaclonal variation. However, unwanted mutants can be removed. But this is not possible for perennial plants.
- Some unwanted mutations cannot be recognized immediately and therefore these mutants cannot be removed.
- Somaclonal variation also affects secondary metabolite production in cell cultures (Babaoğlu, M. 2002).

2. Some Studies Conducted with Plant Tissue Culture Techniques

2.1 Determination of Salt Tolerance of Cultivated and Wild Tomato Species by *İn vitro* Shoot Tip Culture Method

Cano A. E. et al. (1998), in their study, measured the salt tolerance of cultured (*Lycopersicon esculentum* Mili.) and wild (*Lycopersicon pennellii* (Correll) D'Arcy) tomato species using the *in vitro* shoot tip culture method and correlated them with the results obtained with callus culture. During the study, they tried to define the developmental and physiological characteristics by adding 0, 35, 70, 105, 140, 175, and 210 mM NaCl to the MS medium they used for shoot tip and callus formation. In their studies, while they could not observe root formation from explants of many cultivated species (*Lycopersicon esculentum* Mili.) even in low salt amounts, they detected root formation from explants of wild species (*Lycopersicon pennellii*) in different salt amounts. When callus fresh weight and shoot fresh weight were compared in different salt amounts, they found that the wild (*Lycopersicon pennellii*) species was more resistant to salt. According to their results, the K^+ ion amount accumulated more in the callus culture of the culture (*Lycopersicon escu-lentum* Mili.) species, and on the other hand, proline aa in the shoot tip culture method. They reported that the amount accumulated more in the wild (*Lycopersicon pennellii* (Correll) D'Arcy) species.

2.2 Determination of salt tolerance of potato varieties by *in vitro* methods

Khrais T et al. (1998), in their study, investigated the salt tolerance of 130 European and North American potato varieties under *in vitro* conditions. In the experiments, plantlets consisting of single node explants cultured in media containing MS + (0, 40, 80, and 120mM) NaCl for 1 month through micropropagation were used. In their research, they evaluated parameters such as shoot and root length, fresh and dry weight of the plantlets formed after 4 weeks. The experiments were repeated twice for each salt concentration and each species. According to the results of the parameters subjected to multi-variation analysis, they classified 130 species into 8 groups in terms of salt tolerance. Accordingly, “Amiks”, “Belrus”, “Bintje”, “Onaway”, “Sierra” and “Tobique” varieties were included in the 1st group and were reported as the species with the most tolerance to salt.

2.3 Determination of salt tolerance in some vine varieties (*Vitis vinifera* l.) under *in vitro* conditions

In 1999, SİVRITEPE N. et al. undertook a study to assess the salt tolerance of Çavuş, Müsküle, and Sultani seedless grape cultivars in a controlled laboratory environment. The researchers propagated plant material using the active bud culture approach in their trials. Individual shoots were subjected to five distinct levels of NaCl concentrations (0.00%, 0.25%, 0.50%, 0.75%, and 1.00%) in a medium containing MS+5 mM BA. This experiment was conducted for two different durations: 4 weeks and 8 weeks. The researchers discovered that the proliferation rate, growth, total chlorophyll amount, and viability of the explants declined as the NaCl concentration and application time increased. Furthermore, it was established that the application of salt resulted in necrosis in the explants. The extent of this damage varied based on factors such as the variety of the explants, the concentration of NaCl, and the duration of the application. According to their report, the Çavuş grape variety exhibited the greatest resistance to salt applications, while the Sultani Seedless and Muscule grape varieties followed closely. The grape varieties that were found to be salt-resistant were able to maintain their growth rates relatively well in salty environments and avoid metabolic disorders like chlorophyll deficiency.

2.4 Research on determining *in vitro* salt tolerance of some potato species cultivated in turkey

Turan M. (2000) tested the salt resistance of 15 potato varieties and 6 wild potato genotypes under *in vitro* conditions. The tested varieties and genotypes were first grown in MS nutrient medium containing 5.12 g/l NaCl, and the tested varieties and genotypes were cultured in medium containing MS+ 2.25 mg/l BAP, 0.2 mg/l NAA and 5 mg/l GA3 for callus formation in salty medium. In this environment, after measuring plant height, plant fresh weight, number of nodes and leaves, leaf width and length, root number and length, it was observed that Obelix, Concorde, Ausonia and Tomensa varieties were more resistant to salt than the other varieties tested. Then, different concentrations of NaCl, MgCl2 and CaCl were added to the nutrient medium of these four varieties. tested the responses to different SAR and EC values obtained by adding salts. As a result of these experiments, Tomensa variety was the best growing variety at different SAR and EC values in terms of plant height, plant fresh weight, number of nodes and leaves, leaf width and length, number and length of roots, and the number of tubers per magenta pot at different SAR and EC values, number of tubers per plant, It was reported that the highest values in terms of tuber weight and callus weight per head were in the Obelix variety, and the highest number of adventitious shoots per cultured explant was in the Tomensa variety.

2.5 Determination of salt tolerance of bread wheat (T*riticum aestivum* l.) varieties

In his research, AKKEÇECİ Ş (2001) used immature embryos of 4 bread wheat (*Triticum aestivum* L.) varieties (Panda, Seyhan-95, Bal Atilla, Marmara-86) in *in vitro* culture in sterile environment (MS + 2mg 2,4-D/l) by culturing, callus formation rates and callus weight increases were determined. The resulting calli were cultured in liquid MS (MS + 2 mg 2,4-D/1) medium containing NaCl at different rates (0, 1.5, 3, 4.5, 5.5, 7 g/l) as a stress factor, and then the stress cultured in liquid MS medium that does not contain factor. The calli obtained here were exposed to the stress factor once again in solid MS medium containing the same proportions of NaCl, and the weight increase in the calli in all environments and the decrease in callus weight depending on the stress factor were determined. None of the varieties tested showed significant differences in salinity (NaCl) tolerance. However, the decrease in callus weight gain of Seyhan-95 variety in the stress environment containing 1.5 g NaCl/1 and of Panda variety in the environment containing 3 g NaCl/1 was less. By increasing the NaCl doses to 4.5, 5.5 and 7gr/l, the decrease in callus weight gain was over 75% in all varieties. It was determined that there were significant differences between varieties in terms of callus formation rate. It was determined that callus weight gain decreased significantly depending on the types and doses due to the increase in the stress factor. Accordingly, he reported that it may be possible to evaluate the responses of varieties to the NaCl stress factor through callus cultures.

2.6 Effects of salt stress on germination and development of *Phaseolus vulgaris* L.

DÖLEK B (2001) conducted a study using mature seed embryos to examine the reactions of five different culture varieties of *Phaseolus vulgaris* L. (“Akman 98”, “Göynük 98”, “Karacaşehir 90”, “Şehirali 90”, and “Yunus 90”) to salt stress in controlled laboratory circumstances. The plants were grown on MS medium supplemented with different quantities of NaCl (0.0%, 0.6%, and 1.0% w/v NaCl). The quantity of embryos that began to grow on days 3, 4, 7, 9, 10, and 16 after being introduced to a substance was documented, and the proportion of mature embryos that successfully began to grow was calculated. The study revealed that the germination rates of fully developed embryos under salt environments exhibited considerable variations across different cultures. Nevertheless, the presence of salt stress significantly inhibited plant development. Based on the acquired results, “Göynük 98” was identified as the most susceptible variety to salt, whereas “Akman 98” demonstrated high salt resistance. The analysis of total soluble proteins was conducted using SDS-polyacrylamide gel electrophoresis in plants of the “Akman 98” and “Göynük 98” varieties, which were cultivated in both control and salt-treated conditions. Salinity was found to cause a decrease in the total protein concentration. The researcher observed distinct peroxidase isoenzymes in plants of two different types that were cultivated in both normal and NaCl-treated conditions.

2.7 Effect of salt stress on growth and development in different Lisanthus (*Eustoma grandıflorum raf.* shinn) varieties in vivo and *in vitro* conditions

In his work, KÜÇÜKAHMETLER Ö (2003) investigated the physiological reactions of Pure white and Pink Picotee Lisianthus cultivars to salt stress under both *in vitro* and in vivo circumstances. The material utilized in *in vitro* salt applications was propagated by the side bud culture technique. The *in vitro* plants were subjected to salt stress for two distinct durations, 4 and 8 weeks, at varying NaCl concentrations of 0, 1.0, 1.5, and 2.0 dS/m in the first trial, and 0, 2.0, 4.0, and 8.0 dS/m in the second and third trials. The study found that the growth, dry weight, and roots of *in vitro* plants reduced as the concentration of NaCl and the duration of administration increased. The NaCl concentrations utilized in the in vivo trials were applied at two distinct periods: the vegetative stage and the generative stage, under tissue culture conditions. The salt applications involved the use of various concentrations of NaCl, which were applied to the potting soil by a drip irrigation system. The study found that the presence of higher levels of NaCl hindered the growth of plants. This resulted in a drop in many factors such as total chlorophyll, dry weight, leaf relative water content, total protein, and total DNA values. Conversely, there was an increase in turgor loss, total sugar, and proline contents. The impacts of salt stress were apparent at elevated NaCl concentrations and during the vegetative phase of growth and development. Under conditions of elevated salt concentrations, there was an observed rise in leaf temperature, accompanied by a decrease in stomatal conductance and transpiration rate. Salt treatments resulted in the buildup of sodium (Na) in both the above-ground and underground plant organs. However, a higher concentration of Na was discovered in the roots compared to the shoots and leaves. The distinction in salt stress tolerance among different kinds was assessed using the tolerance index and tolerance ratio, which were estimated based on the dry weight of plants in *in vitro* studies. In greenhouse trials, the tolerance index and tolerance rate were determined by evaluating not only the dry weight, but also the levels of total chlorophyll, total protein, and proline in the leaves. Both in high NaCl concentrations and during long-term salt stress, the Pure White Lisianthus variety exhibited greater salt tolerance compared to the Pink Picotee type. The greenhouse trials investigating salt tolerance further confirmed these findings by examining the Na:Ca and K:Na levels. Despite variations among different types, it has been established that Lisianthus can sustain its growth and development by shielding itself from the harmful impact of salt, even in environments with high salt concentrations. The resistance established by Lisianthus cultivars towards salt is attributed to their capacity for osmotic management, achieved through the accumulation of solutes such as sugar and proline in their bodies, as well as their ability to selectively absorb ions through their roots.

2.8 *In vivo* and *in vitro* ınvestigation of the effects of salt stress on some physiological parameters and antioxidant enzyme activities in tomato (*Lycopersicon* sp.)

In his study, Doğan M (2004) sought to identify the key factors that can be utilized in the selection of tomato genotypes that are resistant to salt stress. Additionally, he hoped to showcase the practicality of using *in vitro* testing as an alternative to in vivo salt tolerance tests. There were a total of 19 distinct tomato genotypes used as materials. These genotypes consist of one genotype from the wild tomato species *L. peruvianum* and the remaining genotypes are local genotypes from the *Lesculentum L* species. The research was conducted in three phases. During the pre-selection stage, different genotypes were cultivated using a water-based solution. At the two-leaf stage, a concentration of 150 mM NaCl was introduced. The root and stem lengths, fresh and dry weights, number of leaves and areas, root/stem dry weight ratios, ion amounts of root, stem, and leaf tissues (specifically Na^+, K^+, Cl^-), and the K^+/Na^+ ratio were determined on the 14th and 28th days after salt application. These factors were used to assess the amounts of chlorophyll and malondialdehyde (MDA) in the leaf tissues. Consequently, one wild genotype (*L peruvianum*) and two local genotypes ("TR-68516" and "TR-55711") were chosen as salt resistant, while two local genotypes ("TR-63233" and "H-2274") were chosen as salt sensitive. The latter phases of his research involved studying specific genotypes that were either sensitive or resistant. This was done through experiments conducted in water culture and callus cultures, both in vivo and *in vitro*. The genotypes were exposed to a concentration of 150 mM NaCl for a duration of 10 days. Furthermore, the activity of the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) were assessed, in addition to the observations and analyses conducted during the pre-selection phase.

It was shown that there was notable diversity across the genotypes' responses to salt stress induced by the application of 150 mM NaCl. The levels of Na+, K^+ ions, and the K^+/Na^+ ratio in tissues are crucial indicators of salt tolerance. Additionally, the presence of antioxidant enzyme systems has been found to significantly contribute to salt tolerance. Genotypes with lower Na^+ ion levels, greater K^+ ion levels, and consequently higher K^+/Na^+ ratios were found to be resistant to salt. These genotypes exhibited higher levels of chlorophyll and lower levels of MDA compared to the sensitive genotypes. There was a positive correlation between the quantity of MDA and the quantity of Na^+, and a negative correlation with the quantities of K^+, chlorophyll, and the K^+/Na^+ ratio. It was concluded that MDA serves as a significant indicator for salt damage. He asserted that the detrimental impact of salt stress on the stem exceeded that on the roots. Additionally, he discovered that salt-tolerant genotypes exhibited a higher ratio of root/stem dry weight compared to sensitive genotypes. This finding emerged as a significant metric for assessing resistance to salt stress. Enzyme activity in salt-tolerant genotypes exhibited higher levels compared to sensitive ones, and shown a greater rise relative to their respective controls. According to the paper, the variations in salt tolerance across different genotypes were observed both *in vitro* and in vivo circumstances. The findings from the *in vitro* experiments were consistent with those obtained from the in vivo experiments. This suggests that tissue culture can be a viable alternative to in vivo tests for assessing the salt tolerance of tomatoes.

2.9 Determination of salt tolerance and embryogenic callus formation of sugarcane *in vitro*

GANDONOU CH et al (2005), in their study to test the salt resistance of 3 species (“CP70-321”, “CP65-357”, “NCo310”) sugar cane (*Saccharum* sp.) under *in vitro* conditions, used leaf explants at MS+2,4-D for callus formation. They were cultured in media containing hormone and (0.17, 34, 68 and 102 mM) NaCl for 4 weeks. The fresh weights of the calli formed in the culture medium were measured and the number of embryogenic calli formed was calculated. They found that callus fresh weights decreased significantly with increasing salt concentrations. Accordingly, they reported that (“NCo310”, “CP70-321”, “CP65-357”) cultivars were salt resistant, respectively.

2.10 Effects of salt stress on wheat (*triticum aestivum* l.) tissue culture

ŞEN A (2005) conducted a study to determine the effect of different salt concentrations on “Tekirdağ”, “Pehlivan” and “Flamura-85” wheat varieties *in vitro*. To develop callus from mature embryos (seeds) of the varieties, they were cultured in medium containing MS+2,4-D and (0; 0.05; 0.1; 0.15; 0.2; 0.25 M) NaCl for 28 days. At the end of the 28th day, the number and percentage of callus formed from the cultured explants and the average callus fresh weight were calculated. In addition, the amounts of chlorophyll and water-soluble protein, superoxide dismutase, peroxidase and catalase enzyme activities in the leaves of the plants regenerated in the culture medium were determined. In the study conducted *in vitro*, it was determined that “Flamura-85” variety was the most resistant to salt, salt stress decreased the amount of chlorophyll in the leaves of regenerating plants in tissue cultures of all three wheat varieties, water-soluble protein amounts of “Tekirdağ” and “Pehlivan” varieties decreased compared to the control groups, and an increase was observed in “Flamura-85” variety. Superoxide dismutase, peroxidase and catalase activities, which are antioxidant enzymes in the leaves of regenerating plants, increased in tissue cultures of all three wheat varieties due to salt stress, compared to the control groups.

2.11 Determination of salt tolerance of bread wheat species by callus culture method

Dokuyucu T et al (2005), *in vitro*, 4 bread wheat cultivars (Bal-Atilla, Marmara-86, Seyhan-95 and Panda) were tested on liquid and solid MS medium at different salt concentrations (25.6, 51.3, 77.0, 99.4, 119.7 mM). In their study to measure the reactions in the environment, they stated that there were significant differences between genotypes in terms of callus formation rate and callus weights. They determined that the calli formed in liquid MS medium were heavier than those formed in solid medium, that the Panda species formed heavier callus in liquid MS medium, and that the rate of callus formation decreased with increasing salt rates. They reported that no cultivars could withstand high salt concentrations, and that the Panda was the most resistant among the cultivars.

2.12 Determination of wheat varieties under salt stress by organagenesis and callogenesis methods

In his study, Akhtar N (2006) tried to determine the salt tolerance of four different wheat varieties (“Arz”, “Pak-81”, “LU-26”, and “Pavon”) using callogenesis and organogenesis tissue culture methods. He added 1-2 mg/l 2,4-D hormone to the first two of the 3 different MS media he used for tissue culture studies, and 2 mg/l NAA hormone to the third. Four different salt amounts of 50, 100, 150 and 200 (mM) were added to the 2nd and 3rd media. He subcultured the calli formed in the 1st medium and transferred them to the 2nd and 3rd medium after 4 weeks. He selected salt-tolerant callus lines from these environments and placed them back in the normal regeneration environment (after 4 weeks). According to the results obtained, it was determined that the callus formation and regeneration rates of each species were different in different salt amounts. Accordingly, it was reported that the most salt-resistant species among the variety was “LU-26”, followed by “Pavon”, “Pak-81”, and “Arz” varieties, respectively. It was stated that these results could be helpful in growing salt-tolerant wheat.

2.13 Determination of callus formation and plant regeneration in some rice cultivars under salt stress *in vitro* conditions

Khaleda L et al (2007), in their *in vitro* study, investigated the salt resistance, callus formation and plant regeneration of “HA-1”, “HA-2”, “HA-8”, “Murabajal”, “Gheoch”, “BR-224-2B-2-5” rice cultivars. They conducted a study to define it. In this study, they used MS and LS media containing 2 mg/l 2,4-D hormone and (0.1%, 0.2%, 0.3%) NaCl. They found that all genotypes formed callus in the medium containing 2 mg/l 2,4-D + 0.1% (w/v). They found that among these species, “Murabajal” (38%) showed the highest callus formation rate and Gheoch (23%) showed the lowest rate, BR-224-2B-2-5 (8%) in MS + 0.1% NaCl medium. They reported that increasing NaCl rates had a limiting effect on callus formation and plant regeneration for all species.

2.14 Response of tomato plants exposed to different salt forms and rates

In their study, YOKAŞ et al. (2008) investigated the impact of NaCl, Na_2SO_4, and $CaCl_2$ on the yield, quality, mineral nutrition, and various physiological characteristics of the greenhouse-grown Target F_1 tomato variety. Additionally, they conducted an *in vitro* experiment to assess the effects of NaCl and Na2SO4 on seed and pollen germination. The researchers discovered that seed germination was influenced by both high concentrations of salt and the strength of the MS medium, whether it was full or half strong (1/2 MS). The salt form and doses had an impact on both pollen germination and pollen tube length. The researchers found that pollen germination did not occur when the doses above 50 mM NaCl and 30 mM Na2SO4. In the greenhouse experiment, it was seen that the levels of chlorophyll, stomatal density, plant growth, and yield dropped as the amount of salt increased. The decrease in stomatal density and yield was more noticeable when NaCl was applied. The permeability of the membrane increased with all amounts and types of salt, although the most notable effect was observed with NaCl. The researchers discovered that higher salt concentrations led to the buildup of proline and a drop in the potassium (K) and nitrogen (N) levels in the plant. Additionally, the calcium (Ca) content of the plant fell when treated with sodium chloride (NaCl) and sodium sulfate (Na_2SO_4), but increased when treated with calcium chloride ($CaCl_2$). According to their findings, the decline in plant development and output when exposed to NaCl and Na_2SO_4 stress was caused by a combination of insufficient levels of calcium, potassium, and nitrogen, as well as excessive accumulation of sodium.

2.15 Determination of salt tolerance of sweet potato genotypes under *in vitro* conditions

Dasgupta M et al. (2008), in their study, determined the salt tolerance of 15 sweet potato genotypes *in vitro* and accordingly the antioxidant enzymes (superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GPX, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6) activities. In the studies, 3 different MS samples containing different salt (NaCl) ratios (0, 0.5%, 1%) and NAA (2.7 μM), BA (4.4 μM), GA3 (1.45 μM) and 30 g/l sucrose were used for 8 weeks. They used shoot tips cultured in nutrient medium. In their research, they evaluated growth parameters (number of leaves, number of shoots, number of roots, shoot length, root length) and found that there was a significant decrease in these parameter values with increasing salt concentrations. Accordingly, in their study using the shoot tip culture technique, they stated the salt tolerance of the genotypes in the order: JP 13 > SB-198/1 15 > JO 14 > Gouri > CİP 12 > CİP 13. They reported that antioxidant enzyme activities increased significantly with increasing salt concentrations.

REFERENCES

Akhtar, A. 2006. Callogenesis and Organagenesis Response of Wheat Cultivars under Sodium Chloride Salt Stress. Pakistan Journal Of Biological Sciences 9 (11): 2092-2096.

Akkeçeci Ş. 2001.Bazı Ekmeklik Buğday (*Triticum aestivum L.*) Çeşitlerinin Tuza Toleranslarının Belirlenmesi. KSÜ. Tarla Bitkileri Anabilim Dalı (Yüksek Lisans Tezi) Kahramanmaraş. (In Turkhis)

Almansouri, M., J. M. Kinet and S. Lutts. 2001. Effect of salt and osmatic stresses on germination in durum wheat (*Triticum durum Desf.*). Plant and Soil 231: 243-254.

Babaoğlu, M., Gürel, E., Özcan, S., 2002. Bitki Biyoteknolojisi I: Doku Kültürü ve

Cano E., Perez-Alfocae F., Moreno V., Caro M., C. Bolarin M. 1998. Evaluation of salt tolerance in cultivated and wild tomato species through *in vitro* shoot apex culture. Plant cell, Tissue and Organ Culture 53: 19-26.

Dasgupta, M., Sahoo M. R., Kole P. C.,Muharje A. 2008. Evaluation of orange-fleshed sweet potato (*Ipomoea batatas* L.) genotypes for salt tolerance through shoot apex culture under *in vitro* NaCl mediated salinity stress conditions.Plant Cell Tiss Organ Culture 94: 161-170.

Doğan M. 2004. Domateste *(Lycopersicon Sp.)* Tuz Stresini Bazı Fizyolojik Parametreler ve Antioksidant Enzim Aktiviteleri Üzerindeki Etkilerinin *In Vivo* ve *In Vitro* Olarak İncelenmesi. Hacettepe Üniversitesi Biyoloji Ana Bilim Dalı Doktora Tezi.

Dokuyucu T., Akkeçeci Ş., Akkaya, A., Kara, R. 2005. Investigation of response of bread wheat cultivars to salinity by using callus culture. J Envirol Biol Apr:26 (2): 251-255.

Dölek, B. 2001. Tuz Stresinin *Phaseolus vulgaris* L'nin Çimlenmesi ve Gelişimi Üzerine Etkileri. İ.Ü Biyoloji Ana Bilim Dalı (Yüksek Lisans Tezi) İstanbul. (In Turkhis)

Gandonua, Ch., Abrını, J., Idaomar, M., Skalı Senhajı, N. 2005. Response of sugarcane (*Saccharum* sp.) varietes to embryogenic callus induction and *in vitro* salt stress. AfricanJournal of Biotechnology Vol 4 , pp 350-354,April.

Kaya, D., Kaya, G. ve Kolsarıcı, Ö. 2005. Bazı *Brassica* Türlerinin Çimlenme ve Çıkışı Üzerine NaCl Konsantrasyonlarının Etkileri. Tarım Bilimleri Dergisi 11 (4).

Khaleda L., Ahmed A.M.A. Marzan L.W ve Al-Forkan M. 2007. Identification of callus Induction and Plant Regeneration Responsiveness in Presence of NaCl in in vitro Culture of Some Deepwater Rice. Asian Journal of Plant Science 6 (1): 36-41.

Khrais, T., Leclerc, Y., Donnelly D. J. 1998. Relative Salinity Tolerance of Potato Cultivars Assessed By *In Vitro* Screening. Amer Journal of Potato 75: 207-210

Küçükahmetler, Ö. 2003. Farklı Lısanthus (Eustoma Grandıflorum Raf. Shınn) Çeşitlerinde *In Vivo* ve *In Vitro* Koşullarda Tuz Stresinin Büyümeye ve Gelişime Etkisi. U.Ü Bahçe Bitkileri Ana Bilim Dalı (Doktora Tezi) Bursa.

Munsuz, N. G:Çaycı ve S: Sözüdoğru Ok. 2001. Toprak Islahı ve Düzenleyiciler (Tuzlu ve Alkali Toprakların Islahı) Ankara Üniversitesi Ziraat Fakültesi Yayınları, No:1518, Ankara.

Sadeghian, S. Y. And N. Yavari. 2004. Effect of water-deficit stres on germination and early seedling growth in sugar beet. Journal Of Agronomy and Crop Sciences 190:138-144.

Sivritepe, N., Eriş A. 1999. Determination of Salt Tolerance in Some Grapevine Cultivars (*Vitis vinifera L.*) Under *in vitro* Conditions.Tr. J. of Biology 23:473-485, Tübitak.

ŞEN A. 2005. Buğday (*Triticum aestivum L.*) Doku Kültüründe Tuz Stresinin Etkileri.İ.Ü. Biyoloji Ana Bilim Dalı (Yüksek Lisans Tezi) İstanbul.

Turan, M. 2000. Türkiye'de Kültürü Yapılan Bazı Patates Çeşitlerinin *In vitro*'da Tuza Dayanıklılığının Belirlenmesi Üzerine Araştırmalar. A.Ü.Z.F. Tarla Bitkileri Ana Bilim Dalı Doktora Tezi. (In Turkhis)

Uygulamaları. Selçuk Üniversitesi Vakfı Yayınları, 374s, Konya.

Yaşar, F. 2003. Tuz stresi altındaki patlıcan genotiplerinde bazı antioksidant enzim aktivitelerinin *in vitro* ve *in vivo* olarak incelenmesi. Yüzüncü Yıl Üniv. Fen Bil. Enst. Bahçe Bitkileri Anabilim Dalı (Doktora Tezi) Van 140s.

Yokuş, İ., Levent, T., Bürün, B., Altunlu, H., Altan, F., Kaya C. 2008. Responses of Tomato (*Lycopersicon esculentum* Mill.) Plant to Exposure to Different Salt Forms And Rates. Turk J Agric For Tübitak (32).

CHAPTER 8

ETHICAL SENSITIVITY IN BSc EDUCATION IN PLANT PRODUCTION

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1.Introduction

In Türkiye, although there has not been a significant change in agricultural production areas in the last 20 years, it is often observed that the status of meadows and pastures, which are prioritised for animal production, is changed to non-agricultural purposes. Agricultural land can be used more effectively with the reduction of fallow land and land consolidation studies, which have been accelerated in the last ten years (Anonymous, 2024a).

During the period of 2000 and 2024, although there was no significant change in global and national agricultural areas, agricultural production has been increased. When evaluating the production data for 2024, it is estimated that the production volumes of the cereal group and sugar plants have been higher among the product groups, and that 50% of the total production has been conducted in the Asian continent. Türkiye has been in 8th rank in the world in terms of agricultural production and productivity (Anonymous, 2024a).

Globally, the agricultural production sector has been a higher share of employment in the African continent and underdeveloped countries. The share of the agricultural sector in employment in 2021 was 26.6%, as considered decreasing compared to previous years. The employment in agricultural sector in Türkiye in 2022 was 16% in total employment which is gradually decreasing compared to previous years (Anonymous, 2024a).

Pesticides and plant nutrition products have been widely used to increase yield in plant production. For this aim, American continent has been the production area with the highest regional use of pesticides. While the Netherlands ranks in the first in the use of fertilisers, Türkiye ranks 8th. It was determined that N fertilisers have been used more intensively due to the soil structure of Türkiye and increased rapidly compared to previous production seasons. As a result of global climate change, in order not to be affected by high temperatures and droughts, the irrigation should be done with appropriate methods at the right time, to save water in soil and increase the level of utilisation of plant nutrition products (Anonymous, 2024a).

It can now be considered as knowledge at the level of primary education that both in the world and in Türkiye, arable land is being lost due to erosion caused by inappropriate soil cultivation techniques and stubble burning. It has been reported that agricultural activities contribute 18-25% to greenhouse gas emissions. In 2020, water consumption (withdrawal) for agricultural purposes was reported to be 87.2% (Anonymous, 2024a). Studies have reported that 900-2,000 litres of water are needed to grow 1 kg of wheat, 1,900-4,000 litres to grow 1 kg of rice, 500-1,500 litres to grow 1 kg of potatoes, 2,000 litres to produce 1 kg of vegetables, 11,000 litres for 1 kg of textiles, 15,000-70,000 litres for 1 kg of veal and 3,500-5,700 litres for 1 kg of chicken meat (Schreier, 2002; Yılmaz, 2009).

The concern that plants production activities may be limiting, to varying degrees, the surface and subsurface resources available to humanity and all living things, and the responsibility to reorganise production activities accordingly, is an observation related to ethical sensitivity. Irresponsibility in these matters, affecting producers, consumers and future generations, allow us to think about ethical issues. There are some opinions that these ethical concerns are not sufficiently covered in undergraduate programmes related to plant production (Horticulture, Field Crops, Plant Protection, Biosystems Engineering, Soil Science and Plant Nutrition, Agricultural Biotechnology). It is clear that students' understanding and awareness of ethical issues will contribute to making future plant production practices more sustainable. Addressing ethical sensitivity that may be perceived as problems is of great importance not only for the protection and sustainability of the ecosystem, but also for the welfare of society and future generations. This chapter examines ethical and unethical practices in undergraduate education related to plant production and the possible effects that may be experienced after application, and provides some suggestions to prevent or minimise unethical practices.

2.Protecting the ecosystem

Plant production is a critical part of ensuring sustainable food security and supply. This production involves a range of operations, processes and practices that can have both positive and negative impacts on the environment. The potential negative impacts, consequently, affect the sustainability of natural resources and the health of the ecosystem(s). The problem is how to maintain this health. For this, we should increase the voice of wider segment of society to protect the ecosystem(s) in order to not to be late any more.

Globally, pesticide use was 2 million tonnes and 1.5 kg/ha in 2000, and became nearly 2.6 million tonnes and 1.8 kg/ha in 2020 (Anonymous, 2024a). The pressure of market size, the need to increase yields declined biodiversity.

This has been increased by using pesticides. Conventional plant production methods can result in varying degrees of yield and quality loss due to inadequate control of pests, phytopathogens and weeds, and minimising these losses is a necessity for national and global food security (Table 1). For a variety of reasons, "chemical control" is the preferred and widely used method of controlling organisms that cause losses in plant production. Undesirable effects of chemical applications include contamination of soil, water and the environment by the pesticides used, killing of non-target organisms to varying degrees (bee deaths, phytotoxicity) and development of resistance (e.g. resistant house flies, weeds) against the active ingredients applied to the organisms (Tirkayi et al., 2010). The negative effects of pesticides on human and environment (Özdemir and Kiraz, 2022) have also led to further objections to the use of chemicals. The study of Koçyiğit and Sinanoğlu (2019) showed that insecticides were found in different levels in the water content in February, April and June in Alara Stream, which is one of the water sources of Alanya location, a famous in vegetable and fruit cultivation place in Türkiye. They also reported that a banned active substance was also detected, which is a striking and remarkable result.

Global fertiliser usage in 2020 was reported to be 200.6 million tonnes, of which 55% was used in Asia. Of the total inorganic fertiliser used globally in 2020, 56% is nitrogen, 24% is phosphorus and 20% was potassium. It has been estimated that these fertilisers varied according to the characteristics of the production area. However, nitrogen fertilisers have been more dominant in the market (Anonymous, 2024a). The pressure to increase soil fertility and produce more plants caused the decrease in all plant production areas. In plant production, intensive fertiliser usage or programmes are used primarily to increase yields. However, excessive or inappropriate use of fertilizers, which is not based on the results of soil analysis, can lead to deterioration of soil structure, pollution of water resources by exceeding limits for drinking and use, and malfunctioning of irrigation systems (Table 1) (Akkeçeci and Özkan, 2022; Cüre, 2022). The pollution of clean and drinking water affect human and environmental health by disrupting the balance of water ecosystems. In order not to this event, it will be possible to use effective fertilisers according to the results of soil analysis and thus protect the ecosystems that fertilisers can reach with a more controlled use of fertilisers.

According to reports, the world's irrigated agricultural area in 2020 will be about 347 million hectares (about 288 million hectares in 2000) (Anonymous, 2024a). Plant production is one of the sectors most affected by global climate change and climate crises. It should not be forgotten that the plant production sector competes with other sectors with other uses, such as mining, etc., as well as domestic and industrial uses in terms of "water consumption". Due to the pressures of population growth, urbanisation and global climate change, competition for available water resources in plant production has increased in all respects and is now an unavoidable reality and one of the major issues of our time (Dolcel et al., 2021). Although irrigation is critical to plant production, irresponsible or wild irrigation practices can lead to lower groundwater levels, depletion or exhaustion of water resources, and increased soil salinity (Table 1). When analysing the climate projections, it is expected that especially the high temperature stress and the related drought stress may increase in the Mediterranean and Southern Europe where in Türkiye is located. It has been reported that 20 of the 36 lakes in the Antalya-Isparta-Burdur-Denizli-Afyonkarahisar-Konya provinces (Lakes District) have completely dried up in the last 30 years and the water levels of the other 16 lakes have decreased significantly due to drought stress caused by the shrinking of their water surfaces (Tulan Işıldar & Yalçıner Ercoşkun, 2021). With respect to water usage end shortage, no measures haven been taken. Water scarcity will both negatively affect environmental sustainability and jeopardise clean and accessible water resources for future generations.

Globally, it has been reported that 80% of arable land is degraded and at risk from erosion (Anonymous, 2024a). Soil erosion can be defined as the sweeping away of the fertile layer of soil on the earth due to different and varying levels of physical geographical variables and their effects. The "sweeping" of the soil surface can occur due to natural causes as well as due to anthropogenic effects. (Duman and İrcan, 2022). As a result of increased deforestation and insufficient reforestation, inappropriate and incorrect tillage methods and some faulty practices such as stubble burning and overgrazing can increase soil erosion (Table 1). This situation leads to a decline in soil fertility and to varying degrees of pollution of water resources.

Plant production can contribute to climate change by releasing greenhouse gases into the atmosphere. It has been reported that 25% of global warming is caused by agricultural activities. It can be said that 25% of 25% in the agricultural sector consists of a large number of components, such as animal and production activities, intensive soil cultivation and exhaust gases from the machinery used in soil cultivation, unnecessary and excessive use of plant nutrients, the type of irrigation and the amount of water consumed per unit area, the characteristics of the soil where agricultural processes or plant production are carried out and the organic matter of the soil. CO_2 emissions from different sources, the amount of which is increasing, may increase the severity of droughts and, as a result, there may be problems in finding sufficient irrigation water due to the decrease in available water resources (Yerli et al., 2015). It is clear that climate change will have a negative impact on plant production, as it will adversely affect plant growth biology and biological processes at different levels. The inevitable consequence of this situation can be summarised as "endangering food security and economic instability" (Table 1).

It is very important to equip our students with the ethical sensitivity to protect the ecosystem in which plant production takes place. It is crucial for students to acquire professional knowledge about the environment and living things that may be harmed by the application of which production method and how they are affected, in order to ensure a more ecological production than the current one.

Table 1. Factors affecting the ecosystem and their possible effects

Factors	Possible effects
Pesticide use	The use of pesticides has a negative impact on the environment and human health. It causes soil and water pollution, phytotoxicity, pest resistance and death of non-target organisms.
Fertilizer use	Excessive use of fertilisers can lead to negative changes in soil properties, pollution of water resources and failure of irrigation systems.
Irrigation	Inappropriate irrigation practices reduce groundwater reserves, leading to depletion of water resources and increased soil salinity.
Soil erosion	Improper tillage and practices such as overgrazing can increase soil erosion. This leads to reduced soil fertility and contamination of water resources.
Global climate change	By interfering with plant growth biology, it can have a negative impact on plant yield and quality, threatening sustainable food security and economic stability.

Students' understanding of the factors that affect ecosystems, and their education and awareness of these issues when they graduate, will contribute to making future plant production practices more sustainable and to the well-being of future generations.

3. Conservation of biodiversity

Biodiversity refers to the variety of plant and animal species, ecosystems and genetic resources. This diversity varies according to the functioning and adaptation of ecosystems and the stresses they experience. However, it is known that some plant production practices can pose serious threats to biodiversity. In this context, it is known that some traditional plant production practices used today have a negative impact on biodiversity by degrading soil quality and reducing or contaminating clean water resources. Biodiversity is affected by factors such as monoculture plant production, genetic erosion, resistance of pests to stress factors and invasion of alien species:

Monoculture plant production can be defined as plant farming in that the same or a single plant species is grown continuously in production areas. Large agricultural enterprises, sustainable food security and economic reasons or agricultural subsidies may partially or fully support monoculture plant production. Monoculture plant production can reduce biodiversity. The main reason for this is that production based on a particular plant species or pattern can lead to the partial or complete loss or reduction of other plant species or even other organisms in the area. Similarly, the same plant species may become partially or completely dominant in the area, giving it an advantage in competition with other species. This can threaten native plant species and other organisms in local ecosystems and further facilitate the spread of species considered to be pests of plant production. In both open field and undercover monoculture production, the population of stress-inducing biotic stressors is constantly increasing, and they are constantly exposed to these stressors in the production area or in the soil (Table 2).

The loss of some species in natural vegetation areas where genetic diversity exists for various reasons is known as genetic erosion. As a result of increasing industrialisation and the use of more land for plant production, the natural conservation areas of plant genetic resources are shrinking or becoming unusable. Plant breeding studies and the intensive farming of genetically developed plant varieties will cause genetic erosion (Kumral and İncedayı, 2014). Although genetic resource pools are used in the preparation or revision of breeding programmes, gene pools may be destroyed or genetic erosion may occur intentionally or unintentionally with the new breeding material (Altındal and Akgün, 2015). With the development of more efficient and higher quality varieties as a result of breeding studies, local varieties are almost at the point of non-production. As a result of this situation, the use of new varieties developed and included in the production pattern has an important share in the loss of genetic diversity. Areas where plant genetic resources are found are used for plant production and overgrazing may occur. In arid ecologies where overgrazing is practised, plant genetic resources and vegetation are irreversibly lost as the whole ecosystem is disrupted (Kumral and İncedayı, 2014). The misuse of areas where plant genetic resources are found, especially pastureland, opening them up for settlement or transferring industrial structures to these areas, etc., has led to the extinction of wild species and the reduction of vegetation diversity (Şehirali and Özgen, 1987). In countries that have developed sensitive policies on this issue, plant genetic resources are protected by "in situ" methods (conservation in their natural habitats) and "ex situ" methods (gene banks, tissue cultures, botanical or zoological gardens). As a result of genetic erosion, if primitive and wild forms are not preserved in gene banks, they gradually disappear from their habitats and are no longer found even in the areas where they are recorded. This can lead to a lack of indigenous genetic resources to control possible future disease or insect outbreaks and to develop genotypes resistant to various stresses such as climate change or crises (Table 2). In order to protect our biodiversity, the opinion of experts (botanist or agriculture engineer) must be considered before land allocations for industrial or public benefit.

It is known that during the migration of human communities, some cultivated plants and some wild species move to the new cultivation area (Nohutçu et al., 2019). If the adaptation of the relocated plants may be high, becoming dominant in these areas, resulting negatively impacts on biodiversity (Table 2).

The World Health Organization defines “the resistance against insecticide” as "the development of the ability of another race of the same insect to tolerate a dose of insecticide that has been shown to kill most individuals in a normal population of a species" (Atmaca Demiröz, 2015). In cases where biotic stress populations can multiply in a short period of time, there has been always a higher risk of 'resistance development', especially if insecticides are used incorrectly or continuously. The continuous and widespread use of pesticides to control biotic stress factors that cause stress in plant production can lead to the development of resistance in harmful organisms (Atmaca Demiröz, 2015). Some of the factors that affect the development of resistance are the reproduction rate of biotic stress, migration, host preference width, sensitivity of other populations to the pesticide, pesticide properties and stability period, application dose, time and frequencies (Table 2). It is clear that the widespread use of pesticides, in addition to the acquisition of resistance by pest organisms, will lead to increased chemical exposure of plants and increased threats to biodiversity.

Biopesticides are defined as formulations produced from naturally occurring substances that can control some biotic stress factors in an environmentally friendly way (Balcı and Durmuşoğlu, 2020). Since the general classification of biopesticides is based on living organisms and natural products, they can be classified as biofungicides (*Trichoderma* spp. etc.), bioherbicides (*Phytopthora* spp. etc.) and bioinsecticides (*Bacillus thuringiensis* etc.) (Mazid et al., 2011). The mechanisms of action of biopesticides are generally environmentally friendly. They have been reported to pose a limited threat to human and environmental health (Table 2), as their toxicity is generally more limited compared to chemical pesticides for the control of important biotic stressors (Mazid et al., 2011; Srijita, 2015). Although the efficacy of biopesticides is slower than their chemical counterparts. Even though their shelf life is shorter, it should be considered that their widespread use is important for both biodiversity and human health.

Decrease of biodiversity can have the negative consequences for both ecological and human health. Therefore, biodiversity should be addressed in undergraduate plant production education. Students should be taught by the courses on biodiversity conservation and sustainability. This will help future agricultural professionals to develop more environmentally friendly and biodiversity sensitive practices. It is also important to promote practices that conserve local plant diversity, such as encouraging the production of local varieties. In this way, plant production can not only ensure food security but also conserve biodiversity.

Table 2. Factors affecting biodiversity

Factors	Possible effects
Monoculture plant production	Monoculture production can reduce biodiversity. It can lead to the disappearance or reduction of plant species other than the main plant. It can threaten native plant species in local ecosystems and facilitate the spread of harmful organisms.
Genetic erosion	Genetic erosion can reduce local plant diversity and lead to the loss of unique plant genes (genes for resistance to various stresses). In the near to medium term, it may be possible to breed plants with more limited resistance to stresses such as disease and climate change.
Cultivation of wild plant species	Cultivated species may become dominant and cause varying degrees of damage to local ecosystems or planting patterns and plant diversity.
Increased use of pesticides and resistance of pest organisms	Pests may develop resistance following the use of pesticides. This can lead to increased use of pesticides and further threats to biodiversity.

By making future agricultural engineers understand the importance of this issue, it will be possible to contribute to the development of biodiversity-sensitive practices. Thus, graduates who encourage the use of local plant seeds will preserve local plant diversity and be capable of protecting biodiversity as well as sustaining food security.

4.Labour rights

Although the plant production sector is an important source of food on a global scale, it is a fact accepted by all parties that it is one of the sectors where the different types of unethical practices can be experienced in terms of workers' conditions. The difficulties experienced by workers in the plant production sector should be considered from both a social and human rights perspective. Some unethical practices affecting farm workers are examined below;

A significant proportion of farm workers in plant production are in precarious employment with low wages. This situation makes it difficult for workers to meet their basic domestic needs, leading to economic insecurity. Seasonal agricultural workers work in plant production for short periods with intermittent basis. This period has not been covered by social security (Table 3). Thus, their low-paid work together with unregistered work is a practice that is far from a humane approach (Akçil and Bayramoğlu, 2022). In Türkiye, the wage provisions have continuously decreased due to the lack or insufficient social security in the seasonal labour force in plant production. On the other hand, there has been a difficulty to find local worker as well. Our agricultural production is nearly based on foreign worker from our neighbour countries. In order to contribute to the prevention of this situation, labour rights and fair remuneration issues should also be emphasised in undergraduate education in plant production. Defending workers' rights and providing humane and fair working conditions should be covered in related courses. In this regard, our students should be advised to empathize when they graduate.

Due to the nature of the work, the plant production sector is often associated with generally and periodically difficult physical working conditions. These conditions vary, but include extremes of heat or cold, long working hours that may be necessary to perform the work, and unsuitable environments. Given the greater likelihood of exposure to sector-specific chemical use (pesticides, plant nutrition, etc.), physical, hygienic and ergonomic risks/risks and hazards (Table 3). There has been a clear need for risk analyses that will inform practices to reduce wages or working hours according to risk (Anonymous, 2024b). With Law No. 6331 on Occupational Safety and Health, workers in plant production have also gained 'legal rights on occupational safety and health'. The main purpose of the legislation is "to prevent plant production workers from being exposed to occupational accidents or diseases, with a protective and preventive approach, to minimise risks and increase welfare", and one of the main objectives is "to increase activities to improve occupational health and safety in the public and agricultural sectors". Main risks; 1) agricultural machinery, 2) physical risk factors (noise, vibration, thermal comfort and light intensity), 3) chemical risk factors (pesticides, gas density, dust, ergonomics), 4) use of drugs, vaccines, etc. (Anonymous, 2024b). All of these factors are of major concern in terms of occupational health and safety. In order to reduce these concerns, it is essential that the courses on occupational health and safety, which are currently attended by students of agricultural faculties to help workers understand and improve safe working conditions, are learnt in such a way that they can be applied in the agricultural enterprises where they will work in the future, and that they direct the personnel they employ to institutions that offer certified courses on this subject, if necessary. In addition, regular inspections should be carried out in agricultural enterprises and there should be a willingness to take the necessary measures to improve occupational safety and to allocate a budget for this, if necessary. Labour rights violations are a common problem in the plant production sector. Today, it has been reported that the estimated number of workers in the agricultural sector worldwide is around 1.3 billion and that 60% of the population in underdeveloped or developing countries (Ağyar and Atış, 2022). A significant proportion of workers in the plant production sector work without a contract with the employer. It has been reported that there are examples where plant production workers do not even know the name of their employer (Ağyar and Atış, 2022). Despite the lack of an employment relationship within the scope of the legislation, it was also reported that a significant proportion of workers in plant production do not have the right to demand that the employer fulfil the obligations arising from a labour contract concluded in accordance with the applicable legislation on issues such as safe working conditions, fair wages, paid/unpaid leave, etc. (Ağyar and Atış, 20-22). The risk of dismissal due to union activities and the fact that there are always masses of migrants willing to work for lower wages instead of workers are important factors that influence the decisions of both employers and workers (Table 3). In this regard, it can only be said that students who will become agricultural engineers should be taught the importance of sweat and work. They should be made aware of the fact that they may have to work with migrants/refugees with a lower profile, who will have to relearn the conditions of the company from scratch and whose safety record they do not know. Their future employees, who are qualified and honest in character, will leave their jobs in the event that unfair practices and will not be given the full value of their labour. In order for all these instabilities to occur in a sustainable manner, there is a need to show the necessary value to local workers, and to provide daily care for agricultural labour, which is a sacred profession that provides sustenance to people, rather than being a degrading job.

Child labour in plant production should be included among the main problems in developing countries. If children have to spend the time necessary for their education and health by working, they cause more or less damage to their future. It is known that the living conditions of children working with their families in seasonal plant production activities are quite inadequate (Table 3). Although it is forbidden to employ children of primary school age, it is known that they have to work with their parents or close relatives for various reasons. Since the family cannot find a safe place to leave their children, they work together with their children in order to finish the work in a short time and to earn more income by staying on the land where plant production is carried out (Ağyar and Atış, 20-22). While boys generally go to work, girls who are old enough to care for their younger siblings contribute to the routine work of the shelter or house (cooking, washing clothes and dishes, caring for the needy, assisting those who go to work) to varying degrees. The environment in which children live in working areas is unhygienic and insufficient in terms of providing basic living needs (Gülçubuk, 2012; Kaya & Özgülnar, 2015; Karadeniz et al., 2021; Ağyar & Atış, 2022). It should be known that in professional agricultural production, everyone who enters the field or farm will be under the responsibility of employers. It should be kept in the minds of future agricultural engineers that the precautions should be taken to provide a suitable environment for children, such as caregiver or service opportunities, if their parents are worked.

The physical and mental development, education and quality of education of children who have to work with their families in plant production is worse than that of children who stay at home. Public awareness about child labour should be increased, legal sanctions should be regulated to prevent the exploitation of child labour with unfair wages, the mental development of these children should be supported with different courses and educational activities, and classes should be opened at different levels to continue their school life during the education period (Kantar Davran et al., 2014; Ağyar and Atış, 2022). In order to solve the problem, attention should be paid to the legislation implemented by the Ministry of National Education (MoNE) (Anonymous, 2024c). Higher education institutions and the Ministries of National Education, Interior and Family should work together to contribute to the prevention of child labour. At the same time, strict inspections of child labour in enterprises and production areas should be carried out and legal regulations should be strengthened.

Although it is a customary and religious practice to "pay the worker for his work before the sweat gets cold", with the ambition to earn more, the payments due to the workers in plant production activities are delayed and even the advance payments from the field or garden owner by the "sergeant/uncle", as the seasonal workers are popularly called, are kept waiting and not paid to the workers on time. In addition, it is also known that the sergeant/uncle takes about one day's wages from the workers every month as a commission, and in addition to this situation, it is also known that they also write a wage for themselves in addition to the sergeant/uncle's wage. In this situation, the plant workers are more or less harmed and victimised. The vehicles used to transport plant workers to the field or garden are not subject to inspections similar to "school buses". In order to prevent this situation, sensitivity should be shown in the implementation of the regulations of the Ministry of the Interior.

Labour rights and conditions must be taken into account in plant production education. Future agricultural professionals should learn to respect workers' rights and fair labour practices. This will both improve the quality of life of workers and contribute to the creation of an ethical agricultural sector. Finally, this situation will reflect positively on productivity as "abundance or productivity".

Table 3. Key ethical issues related to workers' rights

Factors	Possible effects
Low wages	Low wages make it difficult for workers to meet their basic needs and can lead to economic insecurity.
Unsuitable working conditions	The plant production sector generally involves physical working conditions. Excessive heat/cold, chemical exposure and other hazards faced by workers are of concern in terms of occupational health and safety.
Job insecurity	Job insecurity makes it difficult for workers to meet their basic needs and can lead to economic insecurity. Union activities and repression of labour rights defenders can make it difficult for workers to be heard.
Child Labour	When children are forced to spend time at work that is necessary for their education and health, they damage their future.

Future plant production professionals should be committed to respecting workers' rights and to fair labour and remuneration practices. This will improve the quality of life of workers, fulfil social and ethical responsibilities and contribute to the development of the sector.

5.Genetically modified plants (GMPs)

GMPs are used in plant production to ensure food security and increase productivity. Genetic engineering is a technology that aims to alter the genetic structure of plants for a specific purpose. This technology allows the development of more efficient and higher quality varieties that are resistant to abiotic and biotic stresses. However, there are some concerns about genetically modified plants.

GMPs have the potential to spread into the natural environment (Demirhan, 2017). The negative effects of the cultivation of these plants on biodiversity are 'damaging to non-target organisms, gene escaping (uncontrolled wild pollination / hybridisation), emerging of super wild plants, contaminating of soil and water resources, and gaining resistance to stress factors' (Table 4) (Arvas, 2017).

There are some concerns about the effects of eating genetically modified plant products on human health. Allergy is one of the most controversial issues. If the gene that codes for the allergic property of one nutrient is transferred to another nutrient, the allergic property of that nutrient may occur. Important health problems can occur in people with allergies due to the consumption of foods that were previously known to be non-allergenic and thought to be safe (Şen and Altınkaynak, 2014). It was reported that soy allergies have increased by 50% with the consumption of transgenic soy in the UK. Also, there was an increase in upper respiratory, skin and eye allergies among those who collect or work in the processing industry of cotton produced in India due to *Bt* toxin (Şen and Altınkaynak, 2014).

Antibiotic resistance genes used for therapeutic purposes can cause the development of resistance to antibiotics currently used for treatment through horizontal transfer to bacteria considered as pathogens in animals and humans (Çetiner, 2010; Şen & Altınkaynak, 2014). The Food and Drug Administration of the United States of America has reported that such a transition will not occur as a result of the digestive system breaking down protein(s) and DNA (Anonymous, 2024d). Mice fed with transgenic maize were assessed for fatty liver and it was reported that the problem increased with increasing dose

(Séralini et al., 2007). On the contrary, in another study conducted for a similar purpose, mice fed Bt maize for 92 days were compared with mice fed conventionally produced maize and detected no significant clinical or toxicological changes (Appenzeller et al., 2009).

It is controversial whether the consumption of GM products can have direct or indirect carcinogenic effects. Studies conducted at Harvard University reported that GM products are no more harmful than conventionally produced products (Norris, 2015). In other word, people studied on GMP have not decided whether GM foods are unhealthy for human health or not (Table 4). Before increasing the voice on this issue, the possible effects of GM products on human health and food safety should be considered in detail when this issue is discussed in BSc Programmes in plant production.

Also, the beneficial effects of genetically modified plants on plant production, animal and human nutrition, especially on poverty must be re-considered. For instance, it should be considered that they may lower the risks related to the application of fewer pesticides, and also, these may decrease the greenhouse gas emissions by using low amount of fuel. Students should be learned all aspects of GMPs rather than one side of this issue. A balanced way and focus should be preferred in BSc education on agriculture with respect to ethical and scientific concept. Thus, more sensitive, tangible and responsible approach to genetically modified plants can be constructed in justice.

There have been some opinions that GMPs may produce genetically similar plants in monoculture production due to the increased resistance to stress factors. This may be a serious threat for biodiversity (Kağıt and Aslan, 2022). In advanced courses on GMPs, sustainable agriculture and biodiversity conservation must be placed in the BSc programmes of agricultural faculties.

Table 4. Possible effects of genetically modified plants

Places of impact	Possible effects
Ecosystem and Biodiversity	GMPs have the potential to invade and become dominant in the ecosystem where they are grown. These plants may affect natural ecosystems by reproducing hybrids with their wild varieties, which may worsened biodiversity and local ecosystems. Ecosystems will lose its biodiversity by the increased genetically modified dominant plants' farming. Plants with the same genetic structure can be easy targets for other diseases and harmful organisms.
Human health	The fact that the possible effects of the consumption of GM foods on human health are still a matter of debate, it should be taken into account that there may be allergic reactions, antibiotic resistance, toxicity concerns, cancer concerns or unknown health risks related to the consumption of these plants.

Issues of concern to undergraduate students should be presented in a balanced way, particularly in terms of benefits and risks.

6.Nanotechnology applications

Nanotechnology is the next generation of technology using only nanoscale building blocks. This technology is defined as "the ability to manipulate a single nanoscale object". Nanotechnology focuses on the characterisation, production and manipulation of objects between 1 and 100 nm in size (İşleyici et al., 2019). The size of the resulting small particles exhibits unique and novel properties with increased surface area, with remarkable potential for practical applications (Mulvaney, 2015). There have been many different applications of nanotechnology such as modifications of existing device physics to entirely new strategies based on molecular self-assembly and the creation of new materials (Kumar et al. 2023). It is known that there is potential for several direct or indirect studies and applications with plants using nanotechnology. These applications include increasing yield per unit area, controlling biotic stresses, making more efficient use of water and resources, and improving food safety. However, there are some concerns about the applications of nanotechnology in plant production:

Nanotechnology can offer different solutions for food safety and quality that can be used for the food sector or produce applicable potentials in agriculture (Table 5). By using nanomaterials, foods can be coated until they are consumed, preserving their shelf life and product characteristics and keeping them fresh for longer periods (İşleyici et al., 2019). It is possible to contribute to the prevention of the development of both plant and human pathogens (Gökmen and Kışla, 2022). This contribution is based on the principle of creating an antimicrobial surface (Song et al., 2020). In addition, by using nanomaterials as packaging material, it can reduce oxygen permeability and reduce food safety risks by prolonging the spoilage times of foods (Kılınç et al., 2020).

The shelf life and quality losses of foods can be monitored by using smart label, biosensor and nanosensor technology (Baysal, 2020).

Nanoadditives', defined as nano-additives, can provide flavour, preservation, colour and dispersion of special additives and faster absorption in the product to which they are added (Kılınç et al., 2020). By using nanotechnological products, it is possible to develop new products or existing product ranges, improve the taste and physical structure of consumed products in the desired direction, and increase their quality and nutritional value. Vitamin(s), mineral(s) or other nutrients can be added to target foods by using nanotechnology (Table 5). This can increase the nutritional value of foods and reduce nutritional deficiencies. Iron, silver, calcium, selenium, silica and magnesium can be used in the food sector in nanodimensions (Kılınç et al., 2020).

Bioactive components encapsulated with nanotechnology will increase the stability of foods by improving bioavailability (Kılınç et al., 2020).

In addition to the use of nanotechnology applications to improve food safety and quality, it is also clear that there has been a need for more scientific studies and the development of legislation on the adverse effects of nanotechnology applications on human health and the environment.

There are approaches that nano-enabled products, such as nanopesticides and nanofertilisers, are 'environmentally friendly' (Arora et al., 2022). Nano-sized pesticides are known to be more effective at controlling harmful organisms, and nano-sized fertilisers are known to be more effective in releasing plant nutrients. Although nanopesticides are said to be more effective and less toxic than their conventionally ones, there are some concerns about their (eco)safety (Ale et al., 2023). Therefore, there has been a need to further elucidate the environmental impact of nanopesticides and fertilisers, their toxicity and effects on soil microorganisms, aquatic and terrestrial model organisms (Table 5).

It has been reported that there was a need to develop new nanomaterials (Table 5) for gene transfer (Yan et al., 2022).

There is a need to use new technologies to monitor nanotechnological pesticides, fertilisers and packaging materials in food (Table 5). It is therefore important that nanotechnology applications are accepted and supported by public. To ensure the necessary acceptance and support, consumers and all other stakeholders should be confident in all aspects of nanotechnology.

At undergraduate level, courses should include details of how to take steps to reduce ethical concerns on the use of nanotechnology in agricultural products and its traceability (Handford et al., 2014).

Table 5. Areas of application of nanotechnology in plant production and key ethical concerns

Areas of application	**Major ethical concerns**
Improving food safety and quality	The addition of nanomaterials to food, the risks to consumer health and issues such as whether products are correctly labelled should be explained in a balanced way.
Control of biotic stressors and plant nutrition	Issues such as the environmental impact of nanopesticides and fertilisers, toxicity and effects on soil micro-organisms should be explained in a balanced way, raising ethical concerns.
Gene transfer	Ethical and environmental concerns have been raised about the use of genetically modified organisms.
Other uses	There are concerns about human and environmental health issues in plant breeding, efficiency and monitoring in plant production (nanosensors, robots and advanced agriculture), food packaging (nanomaterial-based packaging).

In plant production applications by using nanotechnology, food security, environmental health, labelling of products, social acceptance or concerns, inequalities in global food supply are important parameters to evaluate its acceptability or rejection. In undergraduate education, the positive and possible negative effects of nanotechnology in plant production applications should be taught in a balanced way.

7. Conclusions and Recommendations

Plant production plays a critical role in sustainable food security and economic development on a global scale. However, there have been several ethical issues facing the sector today and in the future. It is also certain that the ethical issues and problems associated with issues such as ecosystem conservation, biodiversity conservation, labour rights, genetically modified plants and nanotechnology will pose major challenges for future agricultural professionals.

The most basic approach to ensuring ethical sensitivity in plant production is to put sustainable agriculture education 'at the centre'. Sustainable agriculture can increase both environmental and social ethical sensitivity. To achieve this;

- At the undergraduate level, students should receive theoretical and practical training in 'good agricultural practices' for soil health, water management, animal production, biodiversity conservation and reduced use of fertilisers and pesticides.
- Another element as important as classroom education is to provide students with field studies and practical experience. Internships in plant production enterprises or organic farms can help students to experience sustainable agricultural practices and to understand the ethical aspects of these practices.
- It is clear that education in sustainable agriculture will make students aware of environmental and social issues. It can help students to understand and be more sensitive to issues such as the negative impact of plant production on the environment, workers' rights, fair distribution of food and contributing to the elimination of food inequality and the needs of local communities.

- The courses on sustainable agriculture can provide agriculture students some opportunities for their research and innovations. Students should be motivated to learn and develop new plant production technologies, tools, methods and practices, and to obtain that these innovations in this area are becoming more sustainable.
- There has been a need for agricultural faculties to collaborate with the agricultural sector, local producers and communities that contribute to plant production. These relations should not only provide students with field experience, but also enable them to better respond to the needs of local producers or the whole sector involved in plant production and to strengthen sustainability efforts. For doing this, their lecturers have been involved directly to organize these collaborations. Thus, the experienced students may participate in field work on plant farms of different sizes, research centres or agribusinesses. These experiences will help students to understand the agricultural sector more comprehensively.
- Sustainable agriculture together with plant and animal production is quite significant to increase ethical sensitivity in agricultural production. This approach can help our undergraduates to be used to be accustomed to the technologies in more responsible and environmentally friendly ways.
- One of the significant methods to focus and awake the ethical sensitivity in plant production is to motivate in making studies and innovations. The use of new technologies and tools can contribute more sustainable and ethically sensitive plant production.
- In BSc education in agriculture, the lecturers should focus to the importance of the biodiversity of our ecosystems.
- Students should organise innovation competitions and activities in faculties with related industry to develop and implement new ideas and products with considering ethical issues and solutions.
- It should be given the opportunities to students to develop communication skills by giving role to them the transferring their research results to related sector in agriculture.

- As seen in Europe, the low-input farming systems can be introduced in teaching and field studies to students by combining plant and livestock production in the same farm. For example, approaches such as beekeeping can contribute to pollination in plant production, or farm manure can improve the soil and help increase plant production. In this way, the negative effects of monoculture agriculture on the ecosystem can be eliminated to some extent. Combining plant and livestock production is an important practice for the agricultural sector. It is clear that the simultaneous production of these two types of products can be beneficial and advantageous for agricultural enterprises.
- Simultaneous plant and livestock production will increase the diversity of products on farms, minimise possible economic risks and prevent possible business losses. Enterprises that produce in this way are more likely to find different markets and increase their income. This method allows the enterprise to contact more customers with a wider trade network.
- As plant and animal production support each other or partially or fully support production, enterprises are expected to reduce various input costs and procurement times. It is possible to reduce existing costs through 'circular resource use' and by using animal waste to support plant production and by using animal waste to support plant production.
- It is clear that the simultaneous production of plants and animals will contribute to the promotion of sustainable agricultural practices. This will contribute to the sustainable use of natural resources more effective manner.

The above recommendations will contribute to the orientation of future agricultural professionals towards more conscious, responsible and environmentally friendly practices. Increasing ethical sensitivity in undergraduate plant production education will help to protect not only producers and agricultural professionals, but also consumers and the natural environment, and the agricultural sector will be able to move towards a more sustainable, ethical and community-oriented future.

References

Ağyar, E., & Atış M. (2022). Mevsimlik Tarım İşçiliğinde Çocukların Maruz Kaldığı Riskler ve Hak İhlalleri. *Türk Kamu Yönetimi Dergisi, 3*(2), 187-193 (in Turkish).

Akçil, M. B., & Bayramoğlu, Z. (2022). Mevsimlik Tarım İşçilerinin Asgari Yaşam Maliyetlerinin Hesaplanması. *Bahri Dağdaş Bitkisel Araştırma Dergisi*, *11*(2), 180-189 (in Turkish).

Akkeçeci, Ş., & Özkan, Ç. Ö. (2022). Organik Tarımda Yeşil Gübre Uygulamasının Önemi ve Sürdürülebilirliği. *Adyutayam Dergisi*, 10(2), 161-174 (in Turkish).

Ale, A., Andrade, V. S., Gutierrez, M. F., Bacchetta, C., Rossi, A. S., Santo Orihuela, P., ... & Cazenave, J. (2023). Nanotechnology-Based Pesticides: Environmental Fate and Ecotoxicity. *Toxicology and Applied Pharmacology*, 116560.

Altındal, D., & Akgün, İ. (2015). Bitki Genetik Kaynakları ve Tahıllardaki Durumu. *Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi*, *12*(1), 147-153 (in Turkish).

Anonim, 2024a. Tarım Sektörü Raporu. Türkiye Bankalar Birliği Haziran 2023, İstanbul 200 sayfa (Date of access 03.01.2024) (in Turkish).

Anonim, 2024b. Tarımda İş Sağlığı ve Güvenliği Rehberi. İş Sağlığı ve Güvenliği Genel Müdürlüğü Politika ve Strateji Daire Başkanlığı. T.C. Çalışma ve Sosyal Güvenlik Bakanlığı Genel Yayın No: 44, 32 sayfa. https://www.csgb.gov.tr/medias/4604/rehber27.pdf (Date of access 03.01.2024) (in Turkish).

Anonim, 2024c. Millî Eğitim Bakanlığı, Mevzuat Bilgi Sistemi ("Mevsimlik Gezici Tarım İşçilerinin Çalışma ve Sosyal Hayatlarının İyileştirilmesi (METİP)" ile "Mevsimlik Gezici Tarım İşçisi Çocuklarının Eğitimleri-Genelge 2011/25") (Date of access 03.01.2024) (in Turkish).

Anonim, 2024d. Agricultural Biotechnology. ISAAA. (2014). http://www.isaaa.org/resources/ publications/agricultural_biotechnology/download/Agricultural_biotechnology.pdf. (Date of access 03.01.2024)

Appenzeller, L. M., Malley, L., MacKenzie, S. A., Hoban, D., & Delaney, B. (2009). Subchronic Feeding Study with Genetically Modified Stacked

Trait Lepidopteran and Coleopteran Resistant (DAS-Ø15Ø7-1xDAS-59122-7) maize grain in Sprague-Dawley rats. *Food and Chemical Toxicology*, *47*(7), 1512-1520.

Arora, S., Murmu, G., Mukherjee, K., Saha, S., & Maity, D. (2022). A comprehensive overview of nanotechnology in sustainable agriculture. Journal of Biotechnology, 355, 21-41.

Arvas Y. E. (2017). *Genetiği Değiştirilmiş Bitkiler ve Tanısı*. Lambert Yayınevi, Düsseldorf, Germany. (in Turkish).

Arvas, Y. E., & Kaya, Y. (2019). Genetiği değiştirilmiş bitkilerin biyolojik çeşitliliğe potansiyel etkileri. *Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi*, *29*(1), 168-177 (in Turkish).

Atmaca Demiröz, D. (2015). Böcekler Neden Direnç Kazanıyor?. *ÇOMÜ Ziraat Fakültesi Dergisi, 3* (2), 91-98. https://dergipark.org.tr/tr/pub/comuagri/issue/25718/27138 (in Turkish).

Balcı, H., & Durmuşoğlu, E. (2020). Bitki koruma ürünü olarak biyopestisitler: tanımları, sınıflandırılmaları, mevzuat ve pazarları üzerine bir değerlendirme. *Türkiye Biyolojik Mücadele Dergisi*, *11*(2), 261-274 (in Turkish).

Baysal, G. (2020). Gıda endüstrisinde nanosistemlerin kullanımı. *Gıda*, *45*(3), 517-529. (in Turkish).

Çetiner, S. (2010). Genetiği değiştirilmiş organizma (GDO) nedir? sorular ve yanıtlar-1. *Uluslararası Ekonomik Sorunlar Dergisi*, *10*(38): 40-54. (in Turkish).

Cüre, B. (2022). Kimyasal ve Organik Gübrelerin Çevre Üzerine Etkisi. *Uluslararası Biyosistem Mühendisliği Dergisi*, *3*(2), 98-107. (in Turkish).

Demirhan, B. (2017). *Patates ve işlenmiş patates ürünlerinde genetiği değiştirilmiş organizmalarla ilgili genetik analizler.* Ondokuz Mayıs Üniversitesi, Fen Bilimleri Enstitüsü Yüksek Lisans tezi, Basılmamış. (in Turkish).

Dolcel, H., Durgun, M., & Gökrem, L. (2021). IoT tabanlı ve makine öğrenmesine dayalı seçici sulama sistemi. *Avrupa Bilim ve Teknoloji Dergisi*, 28, 395-401. (in Turkish).

Duman, N. & İrcan, M. R. (2022). Coğrafi bilgi sistemleri ve uzaktan algılama tabanında Çankırı merkez ilçesinin erozyon risk analizi. *Coğrafi Bilimler Dergisi, 20* (1), 220-245. DOI: 10.33688/aucbd.1074770 (in Turkish).

Erken, S., Özzambak, ME., (2018). *Gentiana cruciata* L. tohumlarının çimlenme özelliklerinin belirlenmesi. V. Ulusal Botanik Kongresi, 11-14 Ekim 2018, Bodrum (in Turkish).

Faydaoğlu, E., & Sürücüoğlu, M. S., (2011). Geçmişten günümüze tıbbi ve aromatik bitkilerin kullanılması ve ekonomik önemi. *Kastamonu Üniversitesi Orman Fakültesi Dergisi, 11*(1), 52-67. (in Turkish).

Gökmen, G. G., & Kışla, D. (2022). Nanoteknoloji Temelli Antimikrobiyal Yüzey Kaplama Teknolojileri ve Potansiyel Uygulama Alanları. *Turkish Journal of Agriculture-Food Science and Technology*, *10*(5), 821-837. (in Turkish).

Gülçubuk, B. (2012). Tarımda çocuk emeği sömürüsü ve toplumsal duyarlılık. *Çalışma ve Toplum*, *2*(33), 75-94. (in Turkish).

Handford, C. E., Dean, M., Henchion, M., Spence, M., Elliott, C. T., & Campbell, K. (2014). Implications of nanotechnology for the agri-food industry: opportunities, benefits and risks. *Trends in Food Science & Technology, 40*(2), 226-241.

İşleyici, Ö., Çakmak, T., Sancak, Y. C., İçek, R., & Tuncay, R. M. (2019). Gıda Ambalajlarında Nanoteknoloji Uygulamaları. Ereğli Uluslararası Bilim ve Akademi Kongresi 9-10 Mart 2019, Cilt 1 pp:171-192 ISBN: 978-605-7852-54-0 (in Turkish).

Kağıt, Y. & Aslan, N. (2022). Tarımda Genetiği Değiştirilmiş Organizmalar Sorunsalları ve Türkiye'nin Durumu. *Lectio Socialis, 6*(1), 23-38. DOI: 10.47478/lectio.992374(in Turkish).

Kantar Davran, M., Sevinç, B.Y., & Seçer, A. (2014). Türkiye'de mevsimlik tarım işçileri çocuklar. *Tarımda iş gücü piyasası,* Erol Yayıncılık, Samsun, Türkiye, 3–5 Eylül 2014 Tutanaklarında Cilt 3, s. 1184–1192 (in Turkish).

Karadeniz, O., Akın, E., & Abacı, N. İ. (2021). Fındık tarımında çocuk işçiliği: Giresun ili Piraziz ilçesi örneği. *Anadolu Tarım Bilimleri Dergisi*, *36*(3): 398-407. (in Turkish).

Kaya, M., & Özgülnar, N. (2015). Mevsimlik (gezici/geçici) tarım işçilerinin iki yerleşim birimindeki yaşam koşulları ve sağlık durumlarına niteliksel bakış. *Türkiye Halk Sağlığı Dergisi, 13*(2): 115-126 (in Turkish).

Kılınç, Y. Sağlam, B., Ay, B., Türk, M., Korkmaz, N., Erengil, Y., Baş, N., Bayık, A. B., & Çomoğlu Naz, H., (2020). Gıda alanında nanoteknoloji. Yıldız Teknik Üniversitesi, Biyomühendislik, BYM4721 Biyomühendislikte Nanoteknoloji, 2020-2021 Güz Dönemi 71 Sayfa. (in Turkish).

Koçyiğit, H., & Sinanoğlu, F., (2019). Yüzeysel sularda pestisit kalıntısının araştırılması çalışma örneği; Alanya Alara Çayı. *Artvin Çoruh Üniversitesi Doğal Afetler Uygulama ve Araştırma Merkezi Doğal Afetler ve Çevre Dergisi, 5* (2): 224-236 (in Turkish).

Kumar, R., Kumar, M., & Luthra, G. (2023). Fundamental approaches and applications of nanotechnology: A mini review. *Materials Today: Proceedings*. https://doi.org/10.1016/j.matpr.2022.12.172 (Baskıda)

Kumral, A., & İncedayı, B. (2014). Gen Kaynaklarının Erozyonu ve Korunması. *Gıda ve Yem Bilimi Teknolojisi Dergisi*, 10: 39-43 (in Turkish).

Mazid S., Kalida J. C. & Rajkhowa R. C. (2011). A review on the use of biopesticides in insect pest management. *International Journal of Science and Advanced Technology, 1*:169-178

Mulvaney, P. (2015). Nanoscience vs Nanotechnology-Defining the Field. *ACS nano*, *9*(3), 2215-2217.

Nohutçu, L., Tunçtürk, M. & Tunçtürk, R. (2019). Yabani Bitkiler ve Sürdürülebilirlik. *Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *24*(2), 142-151 (in Turkish).

Norris, M. L. (2015). Will GMOs hurt my body? The public's concerns and how scientists have addressed them. Science in the News. https://sitn.hms.harvard.edu/flash/2015/will-gmos-hurt-my-body/ Date of access: 10.09.2023

Özdemir, T., & Kiraz, Evci D. E. (2022). Pestisitlerin Çevre Sağlığı Üzerindeki Etkisi. *City Health Journal*, 3(2), 18-23. (in Turkish).

Schreier, H. (2002) Water and agriculture: harvesting the water before harvesting the plant. Workshop and think tank on water and the future of life on earth. Simon Fraser University Morris J. Wosh Centre for

Dialogue. May 22–23, 2002, invited presentation. Proceedings; Chapter 17 Continuing Studies in Science at Simon Fraser University

Şehirali, S., & Özgen, M., (1987). *Bitkisel Gen Kaynakları*. Ankara Üniversitesi Ziraat Fakültesi Yayınları, Ankara, 239 s. (in Turkish).

Şen, S., & Altınkaynak, S. (2014). Genetiği değiştirilmiş gıdalar ve potansiyel sağlık riskleri. *Sakarya Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 18*(1), 31-38. (in Turkish).

Séralini, G. E., Cellier, D., & de Vendomois, J. S. (2007). New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. *Archives of environmental contamination and toxicology*, *52*, 596-602.

Song, B., Zhang, E., Han, X., Zhu, H., Shi, Y., & Cao, Z. (2020). Engineering and application perspectives on designing an antimicrobial surface. *ACS Applied Materials & İnterfaces*, *12*(19), 21330-21341.

Srijita D. 2015. Biopesticides: an ecofriendly approach for pest control. *World Journal of Pharmacy and Pharmaceutical Sciences, 4* (06): 250-265

Tiryaki, O., Canhilal, R., & Horuz, S. (2010). Tarım İlaçları Kullanımı ve Riskleri. *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Fen Bilimleri Dergisi,* 26(2), 154-169. (in Turkish).

Tulan Işıldar, H. & Yalçıner Ercoşkun, Ö. (2021). Göller Yöresinde Sürdürülebilirlik ve Dirençlilik. *Journal of Management Theory and Practices Research*, 2 (2), 89-116. (in Turkish).

Yan, Y., Zhu, X., Yu, Y., Li, C., Zhang, Z., & Wang, F. (2022). Nanotechnology strategies for plant genetic engineering. *Advanced Materials, 34*(7), 2106945.

Yerli, C., Şahin, Ü., Çakmakcı, T., & Tüfenkçi, Ş. (2019). Tarımsal uygulamaların CO_2 salınımına etkileri ve azaltılmasının yolları. *Turkish Journal of Agriculture-Food Science and Technology*, *7*(9), 1446-1456. (in Turkish).

Yılmaz, F. (2009). Su ve Ekosistem. İklim Değişikliği ve Çevre, 2(1), 1-5. (in Turkish).

CHAPTER 9

DETERMINING THE CHARACTERIZATION BY MULTIVARIATE STATISTICAL METHODS OF CHICKPEA FARMS IN CENTRAL ANATOLIA REGION OF TURKIYE

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INTRODUCTION

One of the main areas of interest of policy makers and decision-making units is to examine the supply chain structure of enterprises in the agricultural production process from different perspectives. In scientific studies on supply chain, it can be seen that agricultural enterprises can exhibit heterogeneity due to the technological tools they need and various structural differences. Farm typology and characterization. Clustering studies on agricultural enterprises are very important in the form of understanding the successful functioning of agricultural enterprises, revealing the strengths and weaknesses of enterprises, producing technological solutions and creating appropriate agricultural policies. It is possible to classify agricultural enterprises based on features such as production, cost, marketing-sales channels. Land structure, soil fertility, ecological conditions, use of technology and various socio-economic characteristics are the leading factors that cause heterogeneity in agricultural enterprises. It is seen that agro-ecological factors are used in a significant part of the typology and clustering studies in the literature. It has also been observed that there are very few studies in which economic variables such as production, cost, sales-marketing channel information are used. Multivariate statistical methods emerging as an analytical tool in agricultural economics provide us to create classification, particularly when a high feature dimensional agricultural data is available. In this study, the data collected from chickpea producers were studied. Chickpea is a very important product for Turkish agriculture. While it is an important source of income especially for low-income producers in arid conditions, it is also a source of vegetable protein for low-income consumers. In Figure 1,2,3,4, cultivation areas and production amounts for the last 5 years for Turkiye in general and for the region where this research was conducted are given.

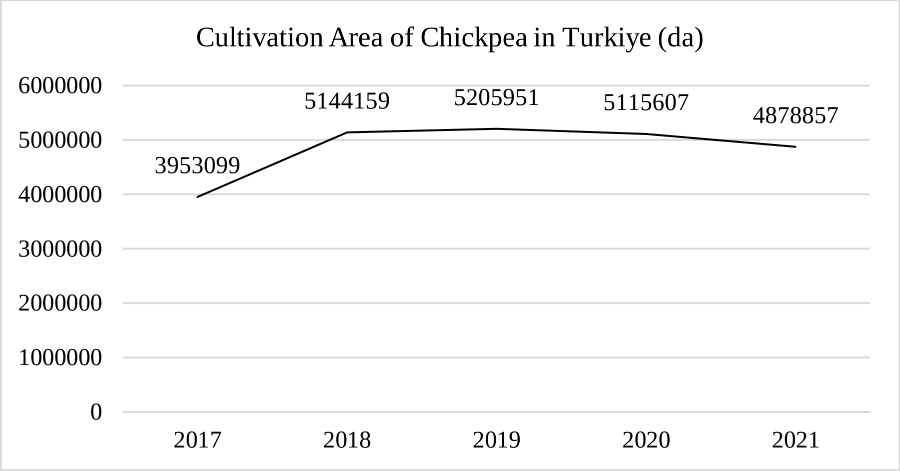


Figure 1. Cultivation Area of Chickpea in Turkiye for last 5 years (Turkish Statistic Institute, 2022)

When the chickpea cultivation areas in the last 5 years in Turkiye were evaluated, it was 3953099 da in 2017 and it was 4878857 da in 2021. Although it increased by 23.47% compared to 2017, it decreased by 4.62% compared to the previous year.

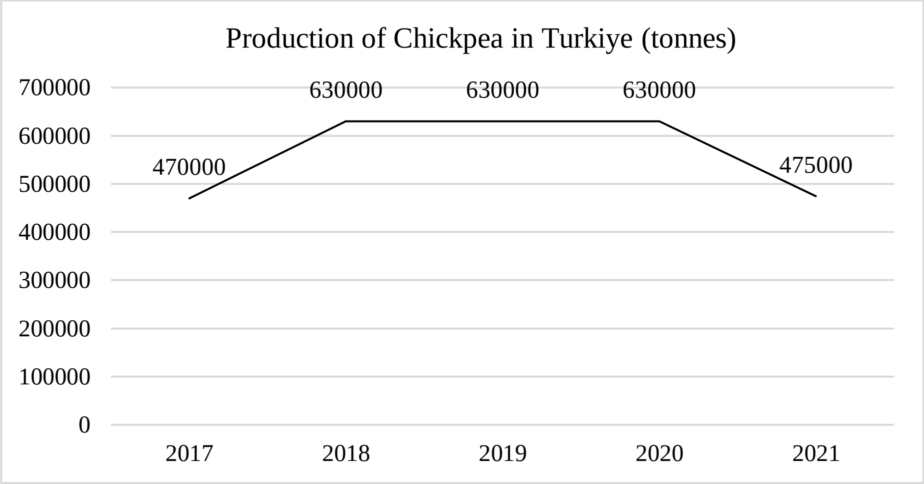
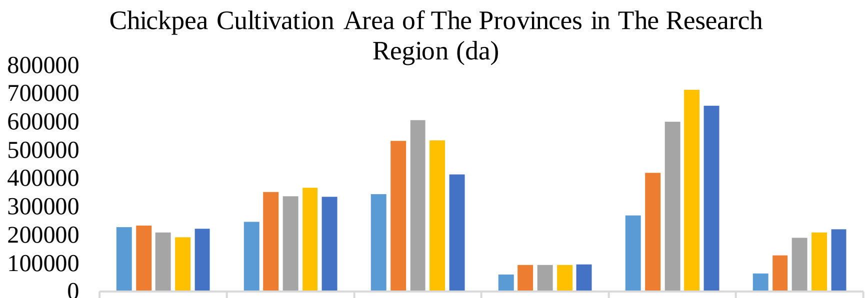


Figure 2. Production of Chickpea in Turkiye for last 5 years (Turkish Statistic Institute, 2022)

When the chickpea production amount for the last 5 years in Turkiye is evaluated, it was 470000 tons in 2017 and it was 475000 da in 2021. Although it increased by 1.06% compared to 2017, it decreased by 24.60% compared to the previous year.



	Karaman	Konya	Kırşehir	Nevşehir	Yozgat	Çorum
2017	227430	246491	343403	59765	268186	64481
2018	232908	351518	532518	94739	418776	128234
2019	208377	336196	606715	93624	600985	189045
2020	191187	366721	535057	94779	712618	209297
2021	222651	334240	413735	95456	657372	220659

2017 2018 2019 2020 2021

Figure 3. Chickpea Cultivation Areas of the Provinces in The Research Region for last 5 years (Turkish Statistic Institute, 2022)

When the chickpea cultivation areas of the provinces in the research region in the last 5 years are evaluated, it can be said that Yozgat has the most cultivation areas. Yozgat province has increased its cultivation areas every year compared to other provinces. However, in the last production year, a shrinkage is observed in the cultivation areas in Yozgat, like the provinces of Konya and Kırşehir.

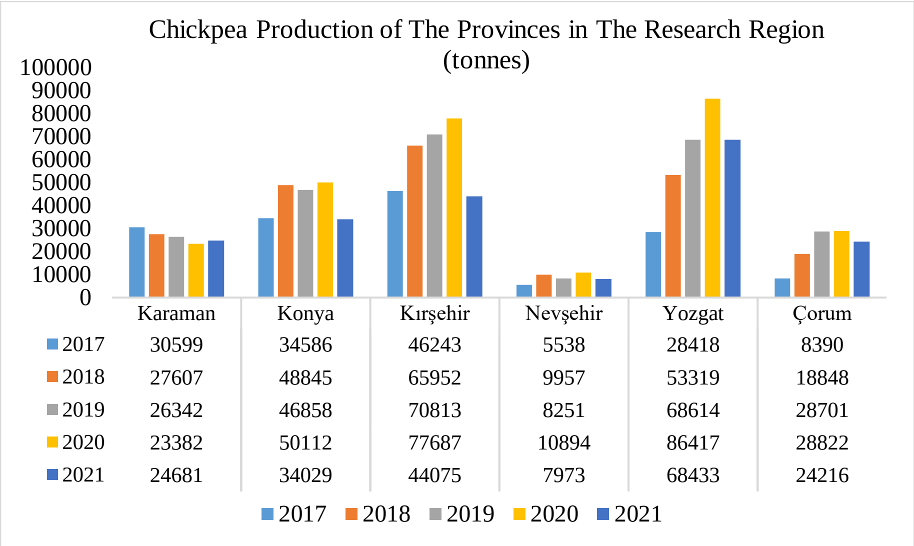


Figure 4. Chickpea Production of the Provinces in the Research Region for last 5 years (Turkish Statistic Institute, 2022)

When the chickpea production amount of the provinces in the research region in the last 5 years is examined, it is not possible to talk about the similar situation in the cultivation areas. Although Çorum, Nevşehir and Karaman provinces have increased in a 5-year trend in terms of cultivation area and especially as of 2021, the last production year, it can be stated that only Karaman province has increased in terms of production amount. This situation can be associated with the climatic factors of the relevant year.

As can be evaluated from the cultivation area and production amounts, different results of the production provinces in the same region can be monitored. It can be stated that this situation is related to farm typologies and local dynamics at the micro level.

In this study, it was tried to determine the characterization of chickpea producers in the provinces of Çorum, Karaman, Kırşehir, Konya, Nevşehir and Yozgat, which are located in the Central Anatolian Region and constitute 39,84% of the chickpea cultivation area in Turkiye. The results of the research play a guiding role for policy makers on chickpea production, which is the most important product of the Project of Narrowing Fallow Lands, which is one of the important livelihoods of the producers in the region. In addition, chickpea is a very important product in Turkiye's agricultural production planning and in bringing the arid lands to agricultural production periodically.

MATERIAL AND METHODS

Study Area and Data Collection; This study was carried out in six provinces, which are constituting of 39,84% chickpea production area of Turkiye, located in the Central Anatolia Region of Turkiye (Çorum, Karaman, Kırşehir, Konya, Nevşehir and Yozgat Provinces). The data were obtained through a comprehensive survey study performed at regional level involving 644 farmers. Distribution of chickpea producers' surveys by province in terms of production amount reported in Table 1.

Table 1. Distribution surveys by provinces in Central Anatolia of Turkiye.

Province	Number of Surveyed Producers	%	Average Production (tonnes)	Standard Deviation
Çorum	80	12.4	10123.85	18669.13
Karaman	68	10.6	4713.514	4760.906
Kırşehir	178	27.6	14364.62	21030.41
Konya	94	14.6	8598.611	18036.09
Nevşehir	42	6.5	2589.881	2418.444
Yozgat	182	28.3	7133.049	10332.93
Total	644	100	9165.512	16066.79

The production regions where the survey study was carried out, were selected among the most relevant provinces in terms of chickpea production in the Central Anatolia region of Turkiye.

Statistical Analysis: In this study, high feature dimensional agricultural data were analyzed by multivariate statistical methods. Multivariate statistical analysis was carried out in two steps: Firstly, PCA was used for examining structural properties of pulse producers. In the second step, factors obtained from PCA were used in K-mean clustering. The Kaiser–Meyer–Olkin (KMO) index (KMO > 0.60) and Bartlett's sphericity test ($p<0.05$) were applied to verify sample adequacy (Bidogeza *et al.,* 2009; Toro-Mujika *et al.*, 2012). The Varimax method was used to rotate the factors (Ibidhi *et al.*, 2018). PCA component with eigenvalues greater than 1 (Kaiser, 1960) were selected for analysis. Two-step clustering approach was used to ensure the stability of clusters. In the first step of clustering procedure, hierarchical cluster analysis was used with Euclidian distance and Ward method. Graphical representation of the hierarchy of nested cluster solutions (dendrogram) which is performed in Ward's method and expert knowledge of pulses producing in the study province were employed to determine an optimal number of clusters. The numbers of clusters obtained from Ward's method were used as a starting value in the K-Means method. Kruskal-Wallis was performed to examine the differences between clusters and in to order to determine the variables how affect the structures of cluster. Also, we applied post hoc test, i.e., Mann–Whitney U-test and Dunn test, which provide us to highlight where the difference is by using multiple comparisons analyses. Multivariate statistical analysis was performed with the statistical software package IBM SPSS 24 and MATLAB R2016.

K-Mean Clustering: The K -Mean clustering method is one of the non-hierarchical clustering methods and is widely used for agricultural economics as in many branches of agricultural science. The K-means algorithm identifies k centroids, one for each of the resulting clusters, and minimizes the objective function based on squared error, thus determining the cluster centers and their associated elements (Tatlidil, 1996; Alpar, 2011; Akilli and Atil, 2020). The focus point in algorithm process is to locate the cluster centers as far as possible from each other and to associate each data point to the nearest cluster center (Ding and He, 2004; Faraoun and Boukelif, 2007). In this study, Euclidean distance is used as the dissimilarity measure. Mathematical representation is given in Equation 1 for the objective function J (Orhan *et al.*, 2011).

$$J = \sum_{i=1}^{K}(\sum_{k}\|x_k - c_i\|^2 \qquad [1]$$

where; K is the number of clusters, $c_i$is the centers of clusters, x_k is kth data point in ith cluster. Where $\|x_k - c_i\|^2$ is a distance measure between the centers of clusters c_i and kth data point x_k. With the implementation of the objective function, clusters are represented by the binary membership matrix U. The elements of matrix U are given in Equation 2 (Orhan *et al.,* 2011).

$$u_{ij} = \begin{cases} 1 \; if \; \|x_k - c_i\|^2 \leq \|x_k - c_t\|^2, \forall t \neq i \\ 0 \quad otherwise \end{cases} \qquad [2]$$

where u_{ij} shows that jth data point belongs to ith cluster, or not. Centroid of cluster c_i is given in Equation 3 (Ding and He, 2004; Faraoun and Boukelif, 2007; Orhan et al., 2011).

$$c_i = \frac{\sum_{j=1}^{N} u_{ij} x_j}{\sum_{j=1}^{N} u_{ij}} \quad [3]$$

The algorithm can be composed of following steps:

1. Data components are assigned a cluster number between 1 and k, this phase is known as the initial phase. Where, k is the number of clusters chosen by the researcher.

2. Cluster centers are calculated. Here, data items are assigned to the group with the nearest centroid.

3. Objective function J operations are performed.

4. The locations of the cluster centers are updated.

5. The process that works after the second step continues until the cluster centers no longer move (Ahmad and Dey, 2007).

RESULTS

K -Mean clustering algorithm with PCA was evaluated to investigate the structural investigations of agricultural enterprises that produce pulses in the Central Anatolia Region in the light of knowledge of production, cost, labor, and marketing strategies. In the study, which was prepared in a very wide and detailed regional basis, it was aimed to provide access to almost all farmers who produce chickpeas. Thus, a high-dimensional and comprehensive sample size was obtained. In order to examine the production structure of 644 agricultural enterprises producing chickpeas, 30 different variables among 200 variables were studied. KMO and Bartlett sphericity tests were applied within the scope of factor analysis. The results show that the KMO indicator is greater than 0.72 and the Bartlett test of sphericity is statistically significant ($p<0.05$). The results show that all variables are related and PCA can be used to obtain the factor structure. According to results of PCA, 10 principal components have been determined (Eigenvalues greater than 1) representing 60.394% of the total variance explained. Eigenvalues and cumulative explained variance information of each principal components were given in Table 2.

Table 2. Eigenvalues corresponding to each principal component (PC) and cumulative explained variance.

Component	Eigenvalues	Cumulative Explained Variance
1	4.239	14.129
2	2.41	22.161
3	2.11	29.194
4	1.954	35.708
5	1.506	40.729
6	1.327	45.153
7	1.266	49.371
8	1.205	53.387
9	1.072	56.961
10	1.03	60.394

Summary of PCA with factor loadings for each of variables was given in Table 3. It is seen in the results of the components obtained as a result of PCA that the variables subject to the analysis form a highly compatible factor structure within the scope of the survey research. All of the components were examined on the basis of the variables which were covered, according to the magnitude of the factor loads formed under them. PCA results reveal that the first component (F1), which explains 14.129% of the variance, is strongly associated with profitability share of the producers, production risk perception of the producers, sale price, cost of top fertilizer and cost of base fertilizer variables. The first component indicates the existence of a positive relationship with other variables except profitability share of the producers. In other words, the increase in fertilizer costs increases the selling price and the risky situation of production. In addition, it has been determined that the producers are negatively affected in the profitability sharing. It was determined that the prominent variables in the elements of the second component were cost of irrigation, cost of harvest-threshing and variation in the last 5 Years and these variables explained 8.032% of the total variance. The second component shows the existence of a positive relationship between cost of irrigation and cost of harvest-threshing and a negative relationship with variation in the last 5 years. The fifth component explains 5.02% of the total variance. The results of the component structure show that variables which are representing the situation of the farmers in supplying seeds in terms of seed quantity and seed variety exhibit a positive relationship. The total variance explained value for the sixth, seventh and eighth components determined within the scope of PCA was calculated as approximately 4%. These components are related to costs and production structure. Finally, the total variance explained value of the ninth and tenth components is about 3%. When all these variables are evaluated together, the total variance explained value was determined as 60.39%. In addition, it can be seen that sales price and cost elements come to the fore among the variables.

Table 3. Principal components derived by PCA with factor loadings for individual crop enterprises and percent cumulative variance explained.

Component Number	% Variance Accumulated	Observed Variables	Factor Loadings
1	14.129% (14.129%)	Profitability Share of The Producers	-0.759
		Production Risk Perception of The Producers	0.734
		Sale Price	0.658
		Cost of Top Fertilizer	0.63
		Cost of Base Fertilizer	0.503
2	8.032% (22.1610%)	Cost of Irrigation	0.843
		Cost of Harvest-Threshing	0.697
		Variation in The Last 5 Years	-0.643
3	7.033% (29.194%)	Farmer Age	0.87
		Farmer Experience	0.853
		Farmer Education	-0.686
4	6.514 (35.708%)	Adequate Equipment	0.752
		Technology	0.738
		Income	-0.567
		Agricultural Risk Perception	0.413
5	5.021 (40.729%)	Seed Variety	0.845
		Seed Quantity	0.827
6	4.424% (45.153%)	Cost of Disease and Pest Control	0.792
		Cost of Total Plough	0.629
7	4.219% (49.371%)	Main Income	-0.639
		Parcel Size	0.628
8	4.016% (53.387%)	Cost of Carriage	-0.746
		Production Yield	-0.412
		Pulses Ratio	0.342
9	3.574% (56.961%)	Man Labor Unit	-0.549
		Cost of Fertilizer Application	0.504
		Information Source on Agricultural Issues	0.424
		Membership to Agriculture Cooperatives/Chambers/Producer Unions	-0.421
10	3.433% (60.394%)	The Proximity of The Marketing Location	0.807
		Cost of Total Sowing	-0.471

Note: Loading values less than 0.3 are ignored.

Distribution of province on to identified clusters are given in Table 4. The distribution of the clusters obtained as a result of the clustering analysis according to the provinces shows that the provinces of Konya and Nevşehir are settled in a balanced way within the cluster structures. It can be interpreted that the mentioned provinces have similar characteristic structures in terms of chickpea production. It has been determined that other provinces are located in more specific locations within the cluster structures. For example, a significant part of the enterprises located in Karaman and Kırşehir provinces are gathered in the fifth and second clusters, respectively.

Table 4. Distribution of province on to identified clusters.

Province	**Producer Cluster**				
	Cluster1	**Cluster2**	**Cluster 3**	**Cluster 4**	**Cluster 5**
Çorum	23	32	4	19	2
Karaman	0	4	0	4	60
Kırşehir	43	85	18	29	3
Konya	23	20	13	17	21
Nevşehir	4	3	20	15	0
Yozgat	30	13	70	67	2
Total	123	157	125	151	88

It was identified that five distinct groups in this population on K- Mean cluster analysis. A total of 123 participants (19.1%) were in Cluster 1, 157 (24.4%) in Cluster 2, 125 (19.4%) in Cluster 3, 1151 (23.4%) in Cluster 4 and 88 (13.7%) in Cluster 5. Characteristics of identified clusters of farms with K-Mean clustering and Kruskal– Wallis test results are shown in Table 5. The Kruskal–Wallis test revealed that differences are statistically significant for almost all analyzed variables, except the farmer age, farmer experience and man labor unit. Based on this result, almost all of the variables in the study were used effectively in the characterization of chickpea producers and in determining the profile pattern of the producers. According to the results in Table 5, when the personal attributes of the farmers are examined, it is seen that only education level of farmers and the main income are statistically significant in the evaluation of the difference between clusters. Numerical results related to personal characteristics show that the main income of the many of farmers in the first cluster is non-agricultural. In addition, it was determined that the farmers in Cluster 1 were at lower levels compared to the other cluster means in terms of parcel size and yield. When the farmer profiles in Cluster 2 are examined, it is seen that the education level is higher, and the man labor unit is higher than the others. In the farmer profiles in Cluster 3, it was determined that the head of the household had a relatively lower education level. In Table 5, it is possible to make evaluations in terms of costs. Here, farmers who were working with lower costs in terms of total plough, irrigation, harvest-Threshing and carriage carried out production activities in larger parcels than others. In addition to these, it can be said that they have a negative attitude in terms of the adequacy of technology and adequate equipment use. It has been determined that the farmers in Cluster 4 have a higher mean of pulses production rate than the others. Finally, farmers in Cluster 5 reported high costs in chickpea production processes. In parallel, they worked with a higher sale price than the farmers in other clusters.

Table 5. Characteristics of identified clusters of farms with K- Mean clustering

Variables	Cluster 1 (N=123)	Cluster 2 (N=157)	Cluster 3 (N=125)	*Cluster 4 (N=151)*	*Cluster 5 (N=88)*	*Kruskal-Wallis*
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean± SD**	**Mean± SD**	χ^2
Personal Attributes						
Farmer Age	49.98±13.39	50.38±11.05	48.25±10.74	46.68±13.11	48.38±10.85	7.53
Education	4.154±1.493	4.25±1.36	3.56±1.22	3.85±1.12	4.18±0.95	22.92*
Main Income	2.00±0.00	1.50±0.50	1.05±0.21	1.00±0.00	1.23±0.42	377.43*
Farmer Experience	25.39±14.86	28.12±12.6	26.09±10.77	26.11±12.88	27.67±10.7	5.25
Man Labor Unit	2.894±1.308	3.05±1.39	2.94±1.26	3.21±1.29	2.76±1.12	8.13
Production Pattern						
Parcel Size	20.53±20.61	29.13±28.29	31.29±39.48	31.23±34.37	24.84±15.54	21.09*
Production Yield	87.5±46.46	101.7±54.9	89.16±49.65	91.27±51.58	136.3±53.05	58.29*
Pulses Ratio	32.99±13.78	34.63±13.37	34.8±14.01	34.85±12.17	22.77±15.42	55.87*
Production Costs						
Total Plough	38.76±25.58	40.40±26.91	30.92±25.23	36.46±27.88	62.77±39.99	45.28*
Total Sowing	116.50±63.73	118.60±70.24	147.1±81.55	130.40±83.02	148.50±41.49	39.49*
Fertilizer App.	1.05±3.78	0.40±2.18	2.585±6.93	1.68±4.40	0.06±0.53	34.41*
Base Fertilizer	18.5±22.9	20.30±23.56	22.77±26.45	16.67±22.83	56.50±22.61	132.36*
Top Fertilizer	2.80±10.27	1.54±6.63	2.18±8.71	0.83±4.70	36.25±16.51	386.43*
Disease and Pest Control	26.91±19.16	32.8±18.09	33.11±22.15	29.09±21.09	33.48±12.09	13.44*
Irrigation	20.85±81.27	3.55±17.92	1.00±8.03	12.27±57.94	82.92±48.2	365.16*
Harvest-Threshing	22.20±5.00	21.19±5.48	20.90±2.70	21.57±4.53	23.3±5.28	27.54*
Carriage	10.18±14.87	9.20±14.63	6.97±11.36	7.81±11.24	13.81±8.05	66.97*
Sale Prices	3.08±0.611	3.24±0.68	2.83±0.59	3.03±0.75	5.47±0.82	223.64*
Reaching Seed						
Seed Quantity	1.27±0.52	1.06±0.26	1.60±0.57	1.34±0.56	1.00±0.00	129.14*
Seed Variety	1.44±0.65	1.07±0.26	1.98±0.70	1.59±0.68	1.00±0.00	204.42*
Profitability Share of The Producers	32.19±23.3	27.96±16.68	32.08±18.6	30.99±17.41	3.64±9.58	171.77*

Table 5 (Cont.). Characteristics of identified clusters of farms with *K*- Mean clustering

Farmer Attitudes						
Agricultural Risk Perception	1.99±0.87	1.08±0.27	2.05±0.91	1.84±0.83	2.18±0.97	139.28*
Technology Level	1.52±0.63	1.22±0.47	1.66±0.72	1.64±0.68	1.08±0.27	85.85*
Adequate Equipment	1.55±0.63	1.20±0.43	1.68±0.66	1.62±0.67	1.16±0.37	81.27*
Income	2.00±0.42	2.14±0.42	2.11±0.46	1.98±0.51	1.98±0.26	16.92*
General Information						
Membership to Agriculture Cooperatives/Chambers/Producer Unions	1.72±0.45	1.00±0.00	1.02±0.15	2.00±0.00	1.03±0.18	514.31*
Information Source	0.18±0.38	0.04±0.21	0.47±0.50	0.11±0.31	0.66±0.48	165.81*
The Proximity of The Marketing Location	21.69±14.06	16.54±11.13	22.28±16.45	18.99±11.13	17.69±12.92	13.89*
Production Risk Perception of The Producers	6.30±1.90	6.45±1.67	6.62±1.66	6.62±2.01	9.41±1.51	143.85*
Variation in The Last 5 Years	3.46±1.25	3.55±1.25	3.62±1.12	3.25±1.18	1.19±0.48	193.20*

* *Statistically significant ($p < 0.05$).*

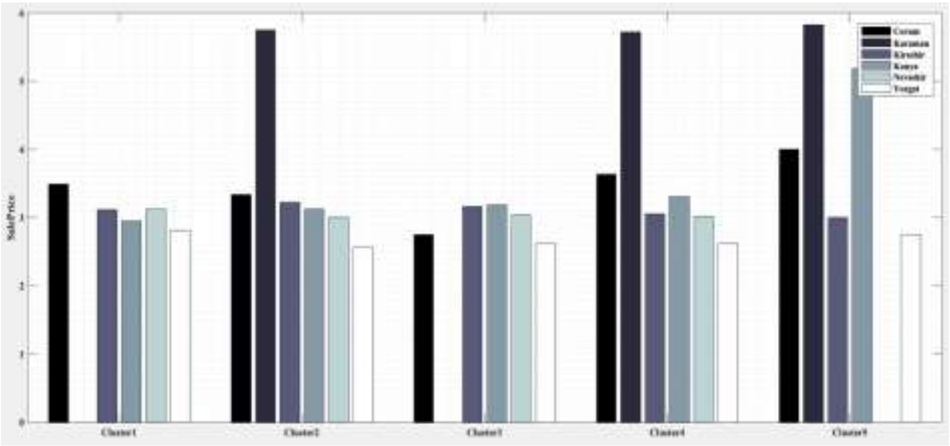


Figure 5. Distribution of identified cluster according to province and sale price**.**

Figure 5 shows the distribution of chickpea sales price according to the defined clusters and provinces in the research region. Accordingly, it was determined that the sales price of chickpeas in Çorum and Konya is higher than in other provinces. It can be said that producer profiles in other provinces have a similar structure in terms of sales prices. When the selling price variable in Table 3 is analyzed independently of Figure 5, it can be seen that the sale prices of the farmers in Cluster 1- Cluster 2 and Cluster 3 are quite close to each other.

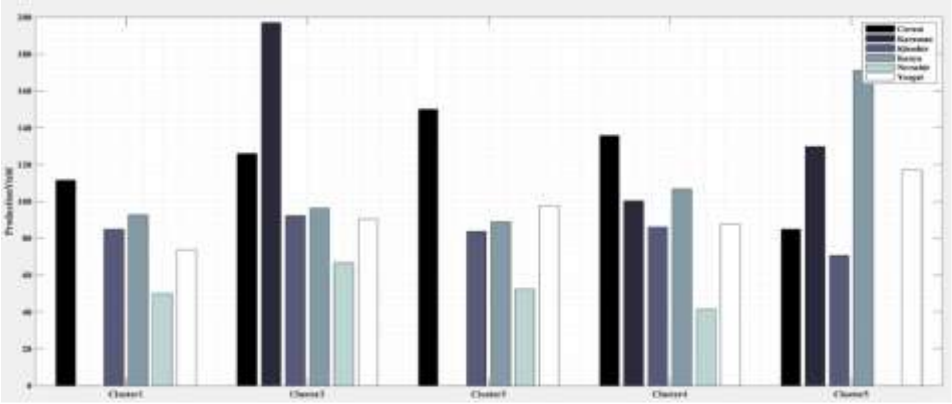


Figure 6. Distribution of identified cluster according to province and production yield.

Figure 6 shows the distribution of yield values according to the defined clusters and the provinces in the research region. Accordingly, all clusters were evaluated together in terms of chickpea yield, and it was determined that Çorum province had higher values compared to other provinces. It was determined as a result of the evaluations made on the basis of clusters that the highest yield value was in the province of Karaman in the second cluster and in the province of Konya in the fifth cluster. When the numerical and graphical results are examined together, it is seen that the profiles of chickpea farmers in the same region have different structures especially in terms of production, cost and sales price.

DISCUSSION

The multivariate approach provided a successful contribution to the characterization of chickpea producers which were examined in terms of supply chain structure. In this study, a set of quantitative and qualitative variable has been used to characterize a sample of 644 chickpea farms located in Center of Anatolia, Turkiye. It is thought that the structures obtained regarding the farmer profiles will contribute to the interpretation of chickpea production behavior and to policy proposals in the future. Principal Component Analysis for reduction of the number of variables followed by Cluster Analysis to characterize farm households has been subjected of many scientific studies (Gebauer, 1987; Jolly, 1988; Hardiman et al.,1990; Solano et al., 2001; Köbrich et al., 2003; Usai et al., 2006, Jansen et al., 2006).

The principal component analysis and the cluster analysis were used by Usai et al., (2006) to characterize the 151 Sardinian goat farms in Sardinia. In the study of Usai et al., 2006, which was carried out to define groups related to regional farm systems, it was clearly shown how diverse goat breeding is, and successful interpretations on development strategies were made similar to our study in economic and social evaluations. Similar results reported by Bidogeza et al., (2009) and Kuswardhani et al., (2014). Bidogeza et al., (2009) used PCA and cluster analysis with Ward’s techniques for distinguishing several farm types in the former Umutara province that might be expected to exhibit different behavior regarding the adoption of technology. As in our results, they focused socio-economic factors. In their study, different types of farm households were identified for providing information source, to understand and diagnose problems. Unlike Bidogeza et al., (2009), in our study, we worked with a much larger data set in terms of the number of variables and the number of observations, but it can be said that harmonious factor structures are formed when socio-economic characteristics are considered. Kuswardhani et al., (2014) have focused the important factors related to adopt greenhouse technology in West Java. For this, in understanding and determining the group-specific problems and opportunities in adoption of new technology, they examined different farm types and used PCA and cluster analysis, as in our study. Different from our study's results, they reported that the prominent variables in factor analysis were area of agricultural farm (ha) and yield (kg/ ha). In our study, the prominent variables in the factor analysis process are related to production risk perception of the producers, cost and sale price, since different geographical regions and production structures are studied. In the literature, there are publications in which livestock farming are subject to cluster analysis (Toro-Mujica et al., 2012; Mena et al., 2016; Todde et al., 2016; Ibidhi et al., 2018). Among these studies, Toro-Mujica et al., (2012) and Ibidhi et al., (2018), similar to our study, applied factor analysis and clustering analysis after preprocessing the data and carried out their research in three basic stages. In our study, we performed similar analyzes with Toro-Mujica et al., (2012) in terms of the number of variables, but it is seen that the explained variance percentage and correlation values were obtained at higher levels than the results of our study. It is thought that the number of observations and the variation in the data set are effective here. Ibidhi et al., (2018), conducted a very high-dimensional data set analysis in their 2018 study and used 25 variables for this. In terms of production and feeding, they evaluated the enterprises they discussed with cluster analysis on a regional basis. Consistent with our study, in the studies of Ibidhi et al., (2018), where the number of observations is quite high, five clusters were obtained for production systems and four clusters for feeding systems.

It is observed from the scientific studies on the typology and characterization of the agricultural enterprises that the factors and cluster structures formed are primarily shaped according to the field of activity.

In the scientific studies on the typology and characterization of agricultural enterprises, it is seen that factor and cluster structures are primarily shaped according to the field of activity. The results of the analysis of scientific studies show that the elements that reveal heterogeneity can basically be examined under some headings. These can be summarized as statistical indicators (number of observations and variables, variation of data structure, details on PCA and clustering analysis), geographical region where agricultural activity is performed, socio-demographic characteristics of farmers, and numerical information on sales-marketing-cost channels of agricultural products, respectively.

CONCLUSION

In this study, it was used cluster analysis to characterization producer patterns and to identify subjects based on the similarity of pulses production. In conclusion, used multivariate statistical analysis methods such as PCA and CA are suitable tools for identifying producer profile for supply chain examination based on socio- economics, production, costs, and farmer attitudes variables. But these methods have some limitations in some situations which are PCA leads to loss of information (Jolliffe, 1986) and cluster analysis has the difficulty of choosing the proper number of clusters especially in high dimensional data set (Alfenderfer and Blashfield, 1984; Everitt et al., 2011). Five different producer profiles and production systems have been defined throughout multivariate approach. The results of the study revealed the heterogeneity of farmer profiles producing in different regions in detail. The results regarding the supply chain structure of the farmers who produce the same agricultural product but exhibit different production behavior in different conditions were obtained. Multivariate analysis methods can be used as a potential tool in agricultural policies to develop strategies and solutions for the sustainability of agricultural production by modeling with numerical values related to important factors such as sales, cost, production, profitability and farmer attitudes. These results showed that the typologies of the farms producing in the same region may be different. This may cause different costs, product quality, sales prices, yield and growing conditions. Therefore, policy makers should consider regional/regional dynamics when planning production, making support policies, and implementing other agricultural policies. According to these differences, the policy tools to be applied will be more effective

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REFERENCES

Ahmad, A., and Dey, L. (2007). A K-mean clustering algorithm for mixed numeric and categorical data. *Data & Knowledge Engineering*. 63, 503-527.

Akilli, A., and Hülya, A. (2020). Evaluation of normalization techniques on neural networks for the prediction of 305-day milk yield. *Turkish Journal of Agricultural Engineering Research*, 1, 354-367.

Alfenderfer, M. S. and Blashfield, R. K. (1984). Cluster analysis. International Professional Publishers Berverly Hills, USA.

Alpar, R. (2011). Çok değişkenli istatistiksel yöntemler. Detay Yayıncılık, 2011.

Bidogeza, J. C., Berentsen, P. B. M., De Graaff, J. and Oude Lansink, A. G. J. M. (2009). A typology of farm households for the Umutara Province in Rwanda. *Food Security*. 1, 321-335.

Ding, C., and He, X. (2004). K-means clustering via principal component analysis. In Proceedings of the twenty-first international conference on Machine learning (p. 29).

Everitt, B. S., Landau, S., Leese, M., Stahl, D. (2011). Cluster analysis, 5th ed. John Wiley & Sons, West Sussex, United Kingdom, 2011.

Faraoun, K. M. and Boukelif, A. (2007). Neural networks learning improvement using the K-means clustering algorithm to detect network intrusions. International Journal of Computer and Information Engineering. 1: 3151-3158.

Gebauer, R. H. (1987). Socio-economic classification of farm households—conceptual, methodical and empirical considerations. *European Review of Agricultural Economics.* 14, 261-283.

Hardiman, R. T., Lacey, R. and Yi, Y. M. (1990). Use of cluster analysis for identification and classification of farming systems in Qingyang County, Central North China. *Agricultural systems*. 33, 115-125.

Ibidhi, R., Frija, A., Jaouad, M. and Salem, H. B. (2018). Typology analysis of sheep production, feeding systems and farmers strategies for livestock watering in Tunisia. Small ruminant research. 160: 44-53.

Jansen, H. G., Pender, J., Damon, A., Wielemaker, W. and Schipper, R. (2006). Policies for sustainable development in the hillside areas of Honduras: A quantitative livelihoods approach. *Agricultural Economics*. 34, 141-153.

Jolliffe, I.T. (1986). Principal components in regression analysis. In Principal component analysis (pp. 129-155). Springer, New York, NY.

Jolly, C. M. (1988). The use of action variables in determining recommendation domains: Grouping Senegalese farmers for research and extension. Agricultural Administration and Extension. 30: 253-267.

Kaiser, H. F. (1960). The application of electronic computers to factor analysis. Educational And Psychological Measurement. 20: 141-151.

Köbrich, C., Rehman, T. and Khan, M. (2003). Typification of farming systems for constructing representative farm models: two illustrations of the application of multi-variate analyses in Chile and Pakistan. *Agricultural Systems*. 76, 141-157.

Kuswardhani, N., Soni, P. and Shivakoti, G. P. (2014). Cluster analysis for classification of farm households based on socio-economic characteristics for technology adoption in agriculture: A case study of West Java province, Indonesia. *J. Food Agric. Environ.* 12, 238-247.

Mena, Y., Ruiz-Mirazo, J., Ruiz, F. A. and Castel, J. M. (2016). Characterization and typification of small ruminant farms providing fuelbreak grazing services for wildfire prevention in Andalusia (Spain). *Science of the Total Environment*. 544, 211-219.

Orhan, U., Hekim, M. and Ozer, M. (2011). EEG signals classification using the K-means clustering and a multilayer perceptron neural network model. *Expert Systems with Applications*. 38, 13475-13481.

Solano, C., León, H., Pérez, E. and Herrero, M. (2001). Characterising objective profiles of Costa Rican dairy farmers. *Agricultural Systems*. 67, 153-179.

Tatlidil H. (1996). Uygulamalı çok değişkenli istatistiksel Analiz. Cem web ofset, Ankara.

Todde, G., Murgia, L., Caria, M. and Pazzona, A. (2016). A multivariate statistical analysis approach to characterize mechanization, structural and energy profile in Italian dairy farms. *Energy Reports.* 2, 129-134.

Toro-Mujica, P., García, A., Gómez-Castro, A., Perea, J., Rodríguez-Estévez, V., Angón, E. and Barba, C. (2012). Organic dairy sheep farms in south-central Spain: Typologies according to livestock management and economic variables. *Small Ruminant Research*. 104, 28-36.

Usai, M. G., Casu, S., Molle, G., Decandia, M., Ligios, S. and Carta, A. (2006). Using cluster analysis to characterize the goat farming system in Sardinia. *Livestock Science*. 104, 63-76.

CHAPTER 10

SUSTAINABLE DEVELOPMENT, FOOD AND BIOTECHNOLOGY

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INTRODUCTION

According to FAO, in 2022, over 900 million people worldwide are experiencing food insecurity, while among children under five suffering from malnutrition, namely stunted 148.1 million, wasted 45 million, overweighted 37 million (FAO, 2023). On the other hand, providing the nutritional demands of the world's population is getting harder every day due to the consequences of climate change and the deterioration of the global climate. Today's mass agro-food production has environmentally adverse effects on soil, water and biodiversity distribution. Thus, sustainability is so essential to preserving the ecosystem and providing enough food for everyone. Sustainable food production is a multi-faceted approach to agriculture and food production, which to develop an environmentally responsible, socially just, and economically feasible production model.

Increasing the effectiveness, employment, and added value of food systems is one of the basic principles of sustainable food practices. It is aimed to add value to agricultural products through the adoption of innovative agricultural techniques, reducing food waste, processing and marketing. It also includes protecting and enhancing natural degradation, conserving water, supporting biodiversity and extractable energy range. Preserving the accumulation of people, communities and ecosystems is also at the centre of sustainable food practices. However, it is also important for management to adapt to innovations, as this requires policies to support sustainable practices and promote responsible consumption.

Biotechnology is a valuable tool with innovative approaches to achieve sustainable development goals. Technological developments, especially in the field of food biotechnology, can produce solutions for food processes regarding sustainability. Among these are to enhance the nutritional quality of foods, to improve the protein sources, to improve fermented products, to increase the shelf-life and to innovate biodegradable compounds for packaging.

Nonetheless, there exists risks and uncertainties, social challenges, and ethical issues surrounding the food biotechnology. For example, the problems regarding biodiversity that arise with conventional agricultural and food system can also arise in biotechnological applications in this field. Or increasing nutritional value of food products by genetic modification may support the better public health, but there are still concerns about their adverse health effects too. At this point, it is important to thoroughly investigate the relationship between biotechnology and sustainable food production, discuss the opportunities and all of the available information, and provide clarity on how to reduce any potential risks.

In this chapter, it is aimed to reveal the relations between sustainability and food systems with the innovations in food biotechnology. The content of the chapter includes description on the concept, explanation the relationship between United Nations Sustainable Development Goals and food systems, and finally, information about the innovations in the field of food biotechnology in terms of sustainable development.

1. CONCEPT AND DEFINITIONS

Although sustainability is used as a term quite often in the literature, the terms sustainability, sustainability and sustainable development can indicate different meanings according to different perspectives. In order to advance the topics to be explained in this section on a certain definitional level, it was necessary to first explain these terms and clarify the definitions used in this section.

The word "sustainability" in the Encyclopaedia Britannica means:

"*the long-term viability of a community, set of social institutions, or societal practice*" (https://www.britannica.com/science/sustainability)

The word "sustainable" or conceptually "sustainability" was first used by Hans Carl von Carlowitz in 1713. He suggetsed "sustainable use" (nachhaltende Nutzung) of forest resources, which means balancing between harvesting old trees and ensuring the replacement them with enough young trees in the forest (Pisani, 2006; Scoones 2007). Thus, the concept of sustainability emerged with "sustainable forestry". However, this concept was not used much until global environmental problems grew. Starting from the second half of the 20th century, after the World War II, increasing environmental problems due to development-oriented industrialization, technological development, rapid economic growth and population growth have brought the concept of sustainability back to the agenda (Waas et al., 2011, Purvis et al., 2019).

On the other hand, the definition of sustainable development was first included in the report, widely known as the Brundtland Report, published by the World Commission on Environment and Development in 1987. According to this report;

"*Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs. It contains within it two key concepts:*

- *the concept of 'needs', in particular the essential needs of the world's poor, to which overriding priority should be given; and*

- *the idea of limitations imposed by the state of technology and social organization on the environment's ability to meet present and future needs*." (Brundtland ,1987)

The concept of sustainability is commonly discussed in three dimensions: economic, social and environmental:

- Economic Dimension: The economic dimension of sustainable development focuses on promoting a viable economy to ensure social welfare. Essentially, this dimension aims to create a self-sustaining economy without depleting natural resources or causing significant ecological damage, thus ensuring future prosperity (Mensah, 2019).

- Social Dimension: The social dimension addresses human well-being, equality and social justice. This dimension aims at the fair distribution of resources and opportunities, reducing inequalities and promoting social inclusion (Rosen, 2020).

- Environmental Dimension: The environmental dimension of sustainable development is related to the protection of natural resources, the continuity of biological diversity and the continuity of the ecosystem. Environmental sustainability includes measures such as preventing excessive consumption of natural resources, combating climate change, reducing carbon emissions and increasing the use of renewable energy resources (Rockström et al., 2009).

In this section there is a need to define the food systems approach included in the content of the chapter. Since the commonly used term "sustainable food" may not cover the complex food production stages from agricultural production to consumer consumption, a more accurate term to use would be "sustainable food systems". According to FAO definition;

"*Food systems encompass the entire range of actors and their interlinked value-adding activities involved in the production, aggregation, processing, distribution, consumption and disposal of food products that originate from agriculture, forestry or fisheries, and parts of the broader economic, societal and natural environments in which they are embedded*." (FAO, 2018)

From this perspective, it is necessary to understand what sustainable food systems are. The concept of sustainable food systems goes beyond the term sustainable food and offers a broader framework that includes food production, distribution, consumption and waste management. Therefore, from a sustainability and food systems perspective, In the definition made by High Level Panel of Experts on Food Security and Nutrition it is defined as;

"*Sustainability refers to food system practices that contribute to the long-term regeneration of natural, social, and economic systems, ensuring that the food needs of the present generations are met without compromising the food needs of future generations*." (HLPE., 2023)

The concept of sustainable food systems is designed to ensure not only the sustainability of food, but also the environmental, economic and social sustainability of all components of food systems. Sustainable food systems are within the framework of 3 dimensions of sustainability it is explained as;

"*A sustainable food system (SFS) is a food system that delivers food security and nutrition for all in such a way that the economic, social and environmental bases to generate food security and nutrition for future generations are not compromised.*

This means that:

– It is profitable throughout (economic sustainability);

– It has broad-based benefits for society (social sustainability); and

– It has a positive or neutral impact on the natural environment (environmental sustainability)." (FAO,2018).

According to Dwivedi et al. (2017), ideal food systems provide adequate and healthy food sources; it supports biodiversity and prevents negative ecological and environmental impacts; ensures farmers' livelihoods, ensures diverse landscape, equitable access to land, water, seeds and other inputs. By the sustainable food systems approach, in addition to ensuring the profitability of food production, economic sustainability can be achieved by fair wages and supporting local economies; social sustainability can be achieved by ensuring access to nutritious food to ensure the well-being of all individuals in the food system; and environmental sustainability can be achieved by protecting natural resources and promoting biodiversity in the process from the production of food to its transportation to the consumer. (UNEP, 2016; FAO, 2018; Lindgren, 2018; McClements et al, 2021). Ericksen (2008) stated that food systems are complex networks that interact with the biophysical environment, economic and social structures. Therefore, sustainable food systems require that each component of these networks be managed sustainably. Godfray et al. (2010) state that sustainable food systems need to develop strategies to reduce food losses and waste, support local food systems, and cope with climate change.

2. UN SUSTAINABLE DEVELOPMENT GOALS CONCERNING FOOD SYSTEMS

The 2030 Agenda for Sustainable Development (UN, 2015) defined a comprehensive framework aimed at global peace and prosperity, underpinned by 17 Sustainable Development Goals (SDGs). These goals are a global action plan to collaboratively address poverty, health, education, inequality, economic growth, climate change and environmental protection among all nations, regardless of their development status (UN, 2015; Shi et al., 2019).

The SDGs have their roots in numerous significant international events and meetings. In 1992, during the Earth Summit in Rio de Janeiro, Agenda 21 was accepted by the representatives from 178 countries, which was an action plan for sustainable development. Following this, during the Millenium Declaration was adopted by the UN member states during the Millennium Summit in 2000. In accordance with this declaration, Eight Millennium Development Goals (MDGs) were developed, which aimed to eliminate extreme poverty by 2015. The process of developing the SDGs was initiated with the 2012 Rio+20 Conference, where Member States approved the "The Future We Want" document. Following a negotiation process on the post-2015 development agenda, the adoption of the 2030 Agenda for Sustainable Development was held at the UN Sustainable Development Summit in

September 2015. This agenda of 17 SDGs (Figure 1) represented the culmination of decades of international efforts towards sustainable development. A total of 169 targets were determined, with a different number for each goal (https://sdgs.un.org/goals).

Figure 1. United Nations Sustainable Development Goals. (https://www.un.org/sustainabledevelopment/)

As explained in the previous section, since food systems involve different actors and activities, sustainable food systems are directly or indirectly related to more than one SDG and target. Following are some SDGs related to food systems, which are briefly described.

SDG 2: Zero Hunger aims "to end hunger, achieve food security, improve nutrition and promote sustainable agriculture" (https://sdgs.un.org/goals). To achieve this goal, enough safe and nourishing food must be available to everyone, especially the most vulnerable. It emphasizes the importance of sustainable food and agricultural practices that increase productivity and production, while protecting ecosystems and climate change (FAO, 2019; UN, 2020).

SDG 3: Good Health and Wellbeing aims “to ensure healthy lives and promote well-being for all at all ages” (https://sdgs.un.org/goals). Nutrition plays a crucial role in achieving this goal, as malnutrition, obesity and diet-related diseases directly impact overall health and well-being. Sustainable food systems are essential to provide diverse, balanced and safe diets that can prevent health problems and improve well-being (WHO, 2018; UN, 2020).

SDG 12: Responsible Consumption and Production aims “to ensure sustainable consumption and production patterns” (https://sdgs.un.org/goals). This goal requires efficient management of natural resources, reduction of food waste, and implementation of sustainable practices in food production and consumption. It aims to enable food systems to minimize environmental impact and contribute to sustainability by promoting the need for sustainable supply chains from production to distribution and consumption (UNEP, 2016; UN, 2020).

SDG 13: Climate Action aims “to take urgent action to combat climate change and its impacts” (https://sdgs.un.org/goals). Food systems both create the effects that cause climate change and are primarily affected by climate change. Sustainable agricultural practices and innovations in food biotechnology can support mitigating climate change by reducing greenhouse gas emissions, increasing carbon sequestration, and promoting resilience to climate-related disruptions (IPCC, 2019; UN, 2020).

SDG 14: Life Below Water and SDG 15 Life on Land aims “to conserve and sustainably use the oceans, seas and marine resources for sustainable development” and “to protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss”

(https://sdgs.un.org/goals). Sustainable food systems contribute to this goal by promoting practices that protect soil health, increase biodiversity, and reduce the ecological footprint of agriculture (FAO, 2019; UN, 2020).

SDG 17 Partnerships for the Goals aims "to strengthen the means of implementation and revitalize the Global Partnership for Sustainable Development" (https://sdgs.un.org/goals). Collaborative efforts between governments, the private sector, academia and civil society are crucial to driving innovation in food systems and scaling sustainable practices (Djekic, et al., 2021).

Sustainable food systems are a complex system that aims to integrate agricultural production, food processing, distribution, consumption and waste management in an environmentally sound, economically viable and socially equitable way. With this integration, sustainable food systems can balance the need for food security with environmental stewardship and social well-being. The role of food biotechnology in these systems is to support and enhance these components through innovative solutions that address specific challenges while considering broader impacts on sustainability.

3. ROLE OF FOOD BIOTEHNOLOGY FOR SUSTAINABILITY

Food biotechnology, as scientific field that uses biotechnological methods in the production and processing of food, plays an important role in the development of sustainable food systems with its innovative applications. It covers the use of traditional biotechnological methods such as fermentation processes, as well as modern biotechnological methods such as enzyme technology, genetic engineering, bioengineering, protein engineering and monoclonal antibody-based methodologies in food production and processing. (Ghoshal, 2018). In this section, the scope has been narrowed by including developments in the field of food biotechnology, rather than biotechnological innovations related with the improving agricultural production techniques.

Genetically modified foods:

One of the most important contributions of food biotechnology is the development of health-supporting foods in human nutrition. The development of genetically modified foods made it possible to increase the nutritional value of food. Biofortification is a process that increases the nutritional content of crops through genetic modification and aims to address malnutrition by increasing levels of essential vitamins and minerals. The most well-known example of this applications is Golden Rice, engineered to produce beta-carotene, the precursor to vitamin A. This genetically modified rice variety was developed to combat vitamin A deficiency in regions where rice is the main nutrient (Beyer et al., 2002; Muthayya et al., 2013). In a study , it was found that mean provitamin A concentrations in genetically modified rice were 89–113% and 57–99% of the estimated average requirement for vitamin A for preschool children in Bangladesh and the Philippines, respectively (Swamy et al., 2019). Another example is the development of crops that also contain high levels of essential micronutrients such as iron and zinc. One study has shown that by bio-iron-fortified rice the overall prevalence of anemia was reduced by 80% in Mexican women (Hotz et al., 2008). Researchers are also conducting studies on increasing the zinc content in corn through both traditional breeding and genetic engineering. According to the study of Rosales et al. (2023), pregnant women and children can meet up to 89% and 100% of their estimated average requirements by consuming maize that has been biofortified with zinc. Biofortified products can help combat nutritional deficiencies that affect millions of people worldwide (Saltzman et al., 2013).

Food Processing Innovations:

Fermentation technologies are actually the emerging point of food biotechnology. However, advances in microbial biotechnology have gone beyond traditional food biotechnology with the development of new fermentation processes to produce alternative proteins such as mycoprotein and cultured meat. *Fusarium venenatum* is used for the fermentation processes of mycoprotein, which is currently commercially available as the brand Quorn foods sold in 16 countries worldwide (Finnigan et al., 2017; Saeed et al., 2023). Producing meat by cultivating muscle tissue from animal stem cells is another advancement and called as cultured meat (Kadim et al., 2015). Although there need to be more improvements to reach a real muscle production via cultured meat technology, it is still promising role in sustainable development by providing sustainable protein sources with a lower environmental footprint compared to traditional animal husbandry (Post, 2012; Chriki and Hocquette, 2020). In addition, enhancing probiotic strains to improve gut health is another example for the improvements in fermentation technology. Engineered *Lactobacillus* and *Bifidobacterium* strains produce bioactive compounds that enhance immune function and digestive health. A study showed that specific strains could significantly increase the production of short-chain fatty acids in the gut, improving overall digestive health (Granato et al., 2010).

Enzyme Technology:

Enzymes are used to increase efficiency and product quality in various food processing applications (Adrio & Demain, 2014). Novel food enzymes such as carbohydrases/glycosidases, proteases/peptidases and lipases have been improved by biotechnological methods (Zhang et al., 2019).

Improved Food Safety and Shelf Life:

Techniques like biopreservation and advanced packaging reduce spoilage and contamination, extending the shelf life of food products and reducing food waste. Biopreservation employs natural or controlled microbiota and their antimicrobial products to extend the shelf life and ensure the safety of foods (Popa et al., 2022). Nisin, a bacteriocin produced by *Lactococcus lactis*, is widely used as a natural preservative in dairy and meat products due to its effectiveness against spoilage and pathogenic bacteria (Field et al., 2023).

The application of biotechnology in food systems has the potential to contribute significantly to the achievement of the UN Sustainable Development Goals (SDGs). However, it also brings with it some challenges and risks that need to be managed carefully. In this regard, the difficulties and risks that may arise can be summarized as follows:

1. Environmental Risks: The long-term environmental impacts of GMO-foods (GMOs) and genetically engineered foods are still not fully understood. Concerns include potential gene flow to non-GM plants and impacts on non-target species (Domingo & Bordonaba, 2011).
2. Economic Inequalities: The high cost of biotechnological research and development may favour large companies over small-scale farmers, potentially leading to economic inequalities in the agricultural sector (Wield, D., 2010). On the other hand, farmers who

adopt GMO crops become dependent on biotechnology companies for seeds and related inputs, reducing their autonomy and increasing their financial risk (National Academies of Sciences, Engineering, and Medicine, 2016).

3. Regulatory and Ethical Concerns: The use of biotechnology in food production raises ethical and regulatory issues, including the labelling of GMO and genetically modified foods and public acceptance of genetically modified foods (Thompson, 2007).
4. Potential Health Risks: Although extensive research has shown that GMOs are generally safe, there are still public concerns about the adverse health effects of having genetically modified foods in their diets (Nicolia et al., 2013; Teferra, 2021).
5. Biodiversity Loss: The production of genetically modified crops can lead to a reduction in agricultural biodiversity and damage the planet's natural resources as traditional and local varieties are replaced by high-yielding GMO varieties (Janet, 2011).

4. CONCLUSION

The integration of food biotechnology into sustainable development strategies holds great potential to address global food security issues and promote environmental sustainability. The United Nations Sustainable Development Goals (SDGs) highlight the importance of sustainable food systems to eliminate hunger, improve nutrition and ensure food security for all. Food biotechnology plays an important role in achieving these goals by increasing product yield, improving nutritional content and reducing environmental impacts with its innovative approaches.

Food biotechnology encompasses a range of techniques such as genetic engineering, biofortification, fermentation technologies and enzyme technology. These innovations have led to the development of drought-tolerant and pest-resistant crops, nutritionally enriched foods, and sustainable agricultural practices. By increasing the efficiency and resilience of food production systems, biotechnology helps mitigate the effects of climate change, preserve natural resources, and reduce dependence on chemical inputs.

The advantages of food biotechnology are important for sustainable development. Increased crop yields and improved nutrient content contribute to food security and public health. Reduction in chemical use, resource efficiency and climate resilience are key factors in promoting environmental sustainability. Moreover, developing sustainable protein sources through fermentation and enzyme engineering reduces the environmental footprint of protein production by offering alternatives to traditional livestock farming.

But the adoption of food biotechnology also brings challenges and potential drawbacks. Environmental risks, economic inequalities, regulatory and ethical concerns, and potential dependence on biotechnology companies must be carefully managed. The long-term effects of genetically modified organisms (GMOs) on ecosystems and human health require ongoing research and monitoring. Ensuring equitable access to biotechnological innovations and addressing public concerns about GMOs are essential for the responsible and inclusive development of food biotechnology.

As a result, food biotechnology offers transformative solutions to create sustainable food systems aligned with the UN Sustainable Development Goals. While the benefits are significant, a balanced and prudent approach is required to address the risks and ethical considerations involved. By encouraging collaboration between scientists, policymakers, industry stakeholders and the public, we can harness the potential of food biotechnology to create a more sustainable, resilient and equitable global food system.

5. REFERENCES

Adrio, J.L.; Demain, A.L. (2014). Microbial Enzymes: Tools for Biotechnological Processes. Biomolecules, Vol: 4, 117-139. https://doi.org/10.3390/biom4010117.

Beyer P., Al-Babili S., Ye X., Lucca P, Schaub P., Welsch R., Potrykus I. (2002). Golden Rice: Introducing the β-Carotene Biosynthesis Pathway into Rice Endosperm by Genetic Engineering to Defeat Vitamin A Deficiency. The Journal of Nutrition, Volume 132, Issue 3, Pages 506S-510S, https://doi.org/10.1093/jn/132.3.506S.

Brundtland G. (1987). Report of the World Commission on Environment and Development: Our Common Future. United Nations General Assembly document A/42/427.

Chriki S and Hocquette J-F (2020) The Myth of Cultured Meat: A Review. Front. Nutr. 7:7. https://doi.org/10.3389/fnut.2020.00007.

Djekic, I.; Batlle-Bayer, L.; Bala, A.; Fullana-i-Palmer, P.; Jambrak, A.R. (2021). Role of the Food Supply Chain Stakeholders in Achieving UN SDGs. Sustainability, 13, 9095. https://doi.org/10.3390/su13169095

Domingo J.L, Bordonaba J.G. (2011). A literature review on the safety assessment of genetically modified plants, Environment International, Volume 37, Issue 4, Pages 734-742, https://doi.org/10.1016/j.envint.2011.01.003.

Du Pisani, J. A. (2006). Sustainable development – historical roots of the concept. Environmental Sciences, 3(2), 83–96. https://doi.org/10.1080/15693430600688831.

Dwivedi S.L., van Bueren E.T.L., Ceccarelli S., Grando S., Upadhyaya H.D., Ortiz R. (2017). Diversifying Food Systems in the Pursuit of Sustainable Food Production and Healthy Diets, Trends in Plant Science, Volume 22, Issue 10, Pages 842-856, https://doi.org/10.1016/j.tplants.2017.06.011.

Ericksen, P. J. (2008). Conceptualizing food systems for global environmental change research. Global Environmental Change, 18(1), 234-245. https://doi.org/10.1016/j.gloenvcha.2007.09.002.

FAO. (2018). Sustainable food systems: Concept and framework. Rome, FAO.

FAO, IFAD, UNICEF, WFP and WHO. 2019. The State of Food Security and Nutrition in the World 2019. Safeguarding against economic slowdowns and downturns. Rome, FAO.

FAO, IFAD, UNICEF, WFP and WHO. 2023. The State of Food Security and Nutrition in the World 2023. Urbanization, agrifood systems transformation and healthy diets across the rural–urban continuum. Rome, FAO. https://doi.org/10.4060/cc3017en

Field D., de Ullivarri M. F., Ross R. P., Hill C. (2023). After a century of nisin research - where are we now?, FEMS Microbiology Reviews, Volume 47, Issue 3, fuad023, https://doi.org/10.1093/femsre/fuad023.

Finnigan T., Needham L., Abbott C. (2017). Chapter 19 - Mycoprotein: A Healthy New Protein With a Low Environmental Impact, Editor(s): Nadathur S. R., Wanasundara J. P. D., Scanlin L., Sustainable Protein Sources, Academic Press, Pages 305-325. https://doi.org/10.1016/B978-0-12-802778-3.00019-6.

Galanakis C, 11 - Food waste valorization opportunities for different food industries, Editor(s): Galanakis C., The Interaction of Food Industry and Environment, Academic Press, 2020, Pages 341-422, https://doi.org/10.1016/B978-0-12-816449-5.00011-4.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., ... & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. Science, 327, 812-818. https://doi.org/10.1126/science.1185383.

Ghoshal G. (2018). Chapter 2 - Biotechnology in Food Processing and Preservation: An Overview, Editor(s): Holban A.M., Grumezescu A. M. In Handbook of Food Bioengineering, Advances in Biotechnology for Food Industry, Academic Press, Pages 27-54, https://doi.org/10.1016/B978-0-12-811443-8.00002-5.

Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G., Faria, J. A. F. (2010). Functional foods and nondairy probiotic food development: Trends, concepts, and products. *Comprehensive Reviews in Food Science and Food Safety*, 9(3), 292-302. https://doi.org/10.1111/j.1541-4337.2010.00110.x.

HLPE (2020). Food security and nutrition: building a global narrative towards 2030. A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security, Rome. FAO.

Hotz C, Porcayo M, Onofre G, García-Guerra A, Elliott T, Jankowski S, Greiner T. (2008). Efficacy of iron-fortified Ultra Rice in improving the iron status of women in Mexico. Food Nutr Bull., 29(2):140-9. https://doi.org/10.1177/156482650802900208.

IPCC, 2019: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [P.R. Shukla, J. Skea, E. Calvo Buendia, V. Masson-Delmotte, H.-O. Pörtner, D. C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen, M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P. Vyas, E. Huntley, K. Kissick, M. Belkacemi, J. Malley, (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, 896 pp. https://doi.org/10.1017/9781009157988.

Carpenter J. E. (2011). Impact of GM crops on biodiversity, GM Crops, 2:1, 7-23, https://doi.org/ 10.4161/gmcr.2.1.15086.

Kadim I. T., Mahgoub O., Baqir S., Faye B., Purchas R. (2015). Cultured meat from muscle stem cells: A review of challenges and prospects. Journal of Integrative Agriculture, Volume 14, Issue 2, Pages 222-233, https://doi.org/10.1016/S2095-3119(14)60881-9.

Lindgren, E., Harris, F., Dangour, A.D. et al. (2018). Sustainable food systems— a health perspective. Sustain Sci 13, 1505–1517. https://doi.org/10.1007/s11625-018-0586-x.

McClements D. J., Barrangou R., Hill C., Kokini J. L., Lila M. A., Meyer A. S.,Yu L. (2021). Building a Resilient, Sustainable, and Healthier Food Supply Through Innovation and Technology. Annual Review of Food Science and Technology, 12:1–28. https://doi.org/10.1146/annurev-food-092220-030824.

Mensah J. (2019). Sustainable development: Meaning, history, principles, pillars, and implications for human action: Literature review, Cogent Social Sciences, 5:1, 1653531, https://doi.org/10.1080/23311886.2019.1653531.

Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE (2013) The Global Hidden Hunger Indices and Maps: An Advocacy Tool for Action. PLoS ONE 8(6): e67860. https://doi.org/10.1371/journal.pone.0067860.

National Academies of Sciences, Engineering, and Medicine. (2016). Genetically Engineered Crops: Experiences and Prospects. Washington, DC: The National Academies Press. https://doi.org/10.17226/23395.

Nicolia, A., Manzo, A., Veronesi, F., & Rosellini, D. (2013). An overview of the last 10 years of genetically engineered crop safety research. Critical Reviews in Biotechnology, 34(1), 77–88. https://doi.org/10.3109/07388551.2013.823595.

Post M.J. (2012). Cultured meat from stem cells: Challenges and prospects. Meat Science, Volume 92, Issue 3, Pages 297-301 https://doi.org/10.1016/j.meatsci.2012.04.008.

Popa, E.E.; Miteluț, A.C.; Râpă, M.; Popescu, P.A.; Drăghici, M.C.; Geicu-Cristea, M.; Popa, M.E. (2022) Antimicrobial Active Packaging Containing Nisin for Preservation of Products of Animal Origin: An Overview. Foods, 11, 3820. https://doi.org/10.3390/foods11233820.

Purvis, B., Mao, Y., Robinson, D. (2019) Three pillars of sustainability: in search of conceptual origins. Sustain Sci 14, 681–695. https://doi.org/10.1007/s11625-018-0627-5.

Rockström, J., Steffen, W., Noone, K. et al. (2009). A safe operating space for humanity. Nature 461, 472–475. https://doi.org/10.1038/461472a.

Rosen M.A. (2020). Chapter 2Sustainability: Concepts, Definitions, and Applications. In: Building Sustainable Cities. Edts. Alvarez-Risco A., Rosen M.A., Del-Aguila-Arcentales S., Marinova D. Pages: 15-26, Springer Cham, https://doi.org/10.1007/978-3-030-45533-0

Rosales A, Molina-Macedo A, Leyva M, San Vicente F, Palacios-Rojas N. (2023). Fresh/High-Zinc Maize: A Promising Solution for Alleviating Zinc Deficiency through Significant Micronutrient Accumulation. Foods. 20;12(14):2757. https://doi.org/10.3390/foods12142757.

Saeed, F., Afzaal, M., Khalid, A., Shah, Y. A., Ateeq, H., Islam, F. et al. (2023). Role of mycoprotein as a non-meat protein in food security and sustainability: a review. International Journal of Food Properties, 26(1), 683–695. https://doi.org/10.1080/10942912.2023.2178456.

Saltzman A., Birol E., Bouis H.E., Boy E., De Moura F.F., Islam Y., Pfeiffer W.H. (2013). Biofortification: Progress toward a more nourishing future, Global Food Security, Volume 2, Issue 1, Pages 9-17, https://doi.org/10.1016/j.gfs.2012.12.003.

Scoones I. (2007). Sustainability, Development in Practice, 17:4-5, 589-596. http://dx.doi.org/10.1080/09614520701469609.

Teferra, T. F. (2021). Should we still worry about the safety of GMO foods? Why and why not? A review. Food Science & Nutrition, 9, 5324–5331. https://doi.org/10.1002/fsn3.2499

Shi, L.; Han, L.; Yang, F.; Gao, L. (2019). The Evolution of Sustainable Development Theory: Types, Goals, and Research Prospects. Sustainability 11, 7158. https://doi.org/10.3390/su11247158.

Swamy B. P. M., Samia M., Boncodin R., Marundan S., Rebong D. B., Ordonio R. L., Miranda R.T., Rebong A. T.O., Alibuyog A. Y., Adeva C. C., Reinke R., MacKenzie D.J. (2019). Journal of Agricultural and Food Chemistry, 67 (28), 7986-7994. https://doi.org/ 10.1021/acs.jafc.9b01524.

Thompson, P. B. (2007). Food biotechnology in ethical perspective. 2nd Edition. Springer Dordrecht. https://doi.org/10.1007/1-4020-5791-1.

UN (2015). Transforming Our World: The 2030 Agenda for Sustainable Development. A/RES/70/1.

UN (2020). Sustainable Development Goals. Retrieved from https://sdgs.un.org/goals.

UNEP (2016) Food Systems and Natural Resources. A Report of the Working Group on Food Systems of the International Resource Panel. Westhoek, H, Ingram J., Van Berkum, S., Özay, L., and Hajer M. Job Number: DTI/1982/PA.

Vermeir, I., Verbeke, W. (2006). Sustainable Food Consumption: Exploring the Consumer “Attitude – Behavioral Intention” Gap. J Agric Environ Ethics 19, 169–194. https://doi.org/10.1007/s10806-005-5485-3.

Waas T., Hugé J., Verbruggen A., Wright T. (2011). "Sustainable Development: A Bird’s Eye View" Sustainability 3, no. 10: 1637-1661. https://doi.org/10.3390/su3101637.

WHO (2018). Global nutrition policy review 2016-2017: country progress in creating enabling policy environments for promoting healthy diets and nutrition. World Health Organization.

Wield, D., Chataway, J., Bolo, M. (2010), Issues in the Political Economy of Agricultural Biotechnology. Journal of Agrarian Change, 10: 342-366. https://doi.org/10.1111/j.1471-0366.2010.00274.x.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., & Murray, C. J. (2019). Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. The Lancet, 393(10170), 447-492. https://doi.org/10.1016/S0140-6736(18)31788-4.

Zhang Y., Geary T., Simpson B. K.. (2019). Genetically modified food enzymes: a review. Current Opinion in Food Science, Volume 25, Pages 14-18, https://doi.org/10.1016/j.cofs.2019.01.002.

Zhang S., Zhu H. (2024). Development and prospect of gene-edited fruits and vegetables, Food Quality and Safety, Vol 8: 1-15, https://doi.org/10.1093/fqsafe/fyad045.

CHAPTER 11

MACHINE LEARNING APPROACH IN CLASSIFICATION OF CROP PRODUCERS

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INTRODUCTION

The integration and efficient use of innovative technologies in agriculture have attracted considerable attention from both academics and policymakers, as these elements are crucial to the effectiveness of agricultural research and extension systems (Bozeman, 2000). The deployment of new technologies, developed in line with the digital era, results in higher returns on investment in research and development. Consequently, this generates a production-driven economic impact and improves rural livelihoods. The analysis of farm typology in various contexts is of practical importance for implementing precise and effective technological interventions. Studies on farm typology recognize that farmers are not a uniform group and face various constraints in their farming decisions based on their resources and lifestyles (Soule, 2001). Ellis (1993) observed that small farmers exhibit consistent internal variations across multiple dimensions. While agricultural enterprises and farmers have unique characteristics, they can be clustered into relatively homogeneous groups. Developing a typology of farm holdings or identifying such groups is essential for a realistic assessment of the constraints and opportunities that farmers encounter. This understanding guides the need for appropriate technological solutions, policy interventions (Ganpat and Bekele, 2001; Timothy, 1994; Vanclay, 2005), and comprehensive environmental assessments (Andersen et al., 2009) to inform relevant authorities. Creating well-directed and target-group-oriented policy measures requires comprehensive and objective grouping of farmers to consider multiple factors that define agricultural businesses, thereby acquiring a multidimensional perspective (Graskemper et al., 2021). Moreover, grouping studies are fundamental for understanding the factors that influence the adoption or rejection of new technologies (Kostrowicki, 1977; Mahapatra and Mitchell, 2001).

Accurate estimations obtained using data and information flow managed correctly in the production process are crucial to success in the global economy and sustainable development framework. Developments in information technologies support the use of new methods in the processing of rapidly increasing large amounts of data. Today, the use of various mathematical models and artificial intelligence methods has become prevalent in interpreting producer behavior in the agricultural field. When machine learning techniques, which is one of the sub-fields of artificial intelligence, are compared with traditional statistical methods, it is seen that very successful results are obtained (Shortridge et al., 2016). In addition, there are many advantages in the interpretation of heterogeneity in the data structure and the analysis of different types and quantities of data structures (Viana et al., 2021).

In the realm of agricultural management research, machine learning techniques, particularly those derived from computational modeling tools, have been the focus of numerous successful investigations. Kung et al. (2016) implemented an ensemble neural network for long-term agronomical yield prediction. Their study provided regional forecasts aimed at aiding farmers in avoiding market supply and demand imbalances exacerbated by variations in harvested crop quality. Vij et al. (2020) explored the integration of Internet of Things (IoT) and machine learning methodologies to automate farm irrigation systems. Graskemper et al. (2021) examined farmer typologies by analyzing a broad range of objective variables related to personal, farm, and contextual characteristics, thereby facilitating the design and communication of effective, target-group-specific policies. The researchers employed cluster analysis based on an extensive farmer survey, which included numerous quantitative variables and concrete data on farms, entrepreneurial activities, and socio-demographic information. Additionally, to mitigate potential researcher bias towards specific topics, they utilized an unsupervised machine learning technique, specifically Partitioning Around Medoids, for clustering the farmers.

Machine learning methodologies have been extensively applied in the classification research of various agricultural products. Koklu and Ozkan (2020) utilized computer vision and machine learning techniques to study the certification of dry bean seeds, aiming to develop a method for achieving uniform seed varieties from crop production, which is typically diverse. Maponya et al. (2020) evaluated the effectiveness of multiple machine learning classifiers for crop type mapping using a series of Sentinel-2 satellite images. Thongcham et al. (2020) developed a machine learning algorithm for classifying oyster mushroom (Pleurotus ostreatus) spawns, measuring the performance of five classifiers: support vector machines, nearest centroid classifier, k-nearest neighbor, deep neural network, and decision trees. Additionally, Chawgien and Kiattisin (2021) worked on creating models for sweetness classification using machine learning approaches, employing eight classification-based techniques.

By leveraging various machine learning algorithms, farm management challenges can be effectively predicted or classified with high accuracy using diverse agricultural data. In our study, we have focused on neural networks, a prominent machine learning method. Neural networks have been successfully implemented in a wide array of Agricultural Economics research. Ghaffarian et al. (2022) conducted a systematic mapping study to review literature on the application of machine learning methods in farm risk management. Their results show a significant rise in the utilization of machine learning techniques, such as deep learning and convolutional neural networks, for managing farm risks in recent years. Gopinath et al. (2021) examined the use of machine learning for forecasting international agricultural trade. Their study highlighted the substantial relevance of machine learning models in predicting trade patterns in both the near- and long-term, outperforming traditional methods that often depend on subjective assessments or time-series projections. Kastens and Featherstone (1996) compared the accuracy of two different models in predicting Kansas farmers' out-of-sample survey responses to risk-related questions. Their research investigated whether a decision maker's responses to subjective agricultural risk questions could be accurately predicted based on relevant characteristics. Jena and Majhi (2018) investigated the application of classification techniques in agriculture, utilizing data related to maize production to categorize and group farmers' behaviors. Moreover, Gallo et al. (2013) employed neural networks to classify olive farms, underscoring the diverse applications of neural networks in agricultural economics. Additionally, various neural network models have been utilized in agricultural economics research, such as regressive models (Stastny et al., 2011), time series predictions (Kohzadi et al., 1996), modeling marketing margins (Richards et al., 1998), and analyzing costs and efficiencies among cooperative, proprietary, and captive fluid milk processors (Erba et al., 1996).

In this study, it was aimed to determine the socio-economic and technical characteristics of the farmers and to classify them into groups of similar structures based on the risk situation of the producers of pulses production. NN classifiers were developed for farmers producing pulses, and the classification performance was compared with the K-Mean clustering technique. The multilayer perceptron (MLP) models are structured with different training algorithms and activation functions. In line with this purpose, it is thought that the results will give an idea to policy makers in designing special policies for different farmer classes and that artificial intelligence-based methods can be used as a policy tool on issues such as risk perception and farm behavior.

MATERIAL AND METHODS

Study area and data collection

This study was carried out in eight different provinces located in the Central Anatolia Region of Turkey. The data were obtained through a comprehensive survey study performed at the regional level involving 1197 farmers from 2019 to December 2020. Distribution of dry bean, red lentil, and chickpea producers' surveys by the province in terms of production amount reported in Table 1.

Table1. Distribution of producers' surveys by province in Central Anatolia of Turkey

Production Type	Province	Number of Producers	%	Average Production (± Std. Error of Mean)
Dry Bean	Karaman	34	2.84	10015±1423.51
	Konya	136	11.3	11368.82±744.91
	Nevşehir	59	4.92	23278.81±2633.77
	Yozgat	21	1.75	4036.19±1223.44
Red Lentil	Diyarbakır	105	8.77	9027.61±703.37
	Şanlıurfa	242	20.2	10852.52±646.5
Chickpea	Çorum	77	6.43	10509.85±2157.04
	Karaman	67	5.59	4664.46±583.90
	Kırşehir	172	14.3	14785.30±1621.87
	Konya	89	7.43	8828.64±1956.51
	Nevşehir	32	2.67	3086.71±455.32
	Yozgat	163	13.6	7835.90±838.79
Total		1197	~100%	

Study analysis

In this study, two different methods are discussed to examine the structural features of the producers based on their risky vision of pulses production. The first method is the K-Mean clustering technique, which is structured together with principal component analysis. The factors obtained with the principal component analysis were used as input parameters both in the K-Mean clustering technique and in the analyzes carried out with neural networks. The aim is to interpret high-dimensional data structures more easily with calculated factor loads. KMO and Bartlett sphericity tests were applied within the scope of factor analysis. Analysis was performed with the statistical software package IBM SPSS 26 and MATLAB R2016. Information on the variables included in the models evaluated within the scope of the study is given in Table 2 for each producer type. These variables that make up the input and output patterns in neural network analyzes are discussed under the headings of personal attributes, production pattern, production costs, sale prices, reaching seed, profitability share of the producers.

K-Mean clustering

Cluster analysis is used to identify homogeneous groups or clusters in the data structure within the framework of variety of techniques and rules (Tatlıdil 1996; Alpar 2011; Akıllı and Atıl, 2020). In analyzes based on defined a set of variables, it is expected that the observations sharing the same cluster or group will exhibit a similar structure, while the observations in different clusters are distant from each other (Goswami et al., 2014).

Table 2. Explanatory Variables (Farm/Farmer Characteristics) Used in the Model

Variable Description (Mean±Std.Dev)	Dried Bean	Red Lentil	Chickpea
Farmer Age	46.73±11.08	49.9±12.42	48.7±12.03
Farmer Education	3.82±1.122	2.91±0.94	4.03±1.289
Main Income	1.2±0.400	1.03±0.18	1.18±0.388
Man Labor Unit	2.71±1.257	4.30±1.47	2.98±1.326
Pulses Ratio	25.97±19.16	38.8±16.6	32.4±13.92
Parcel Size	28.41±11.46	52.5±20.2	23.7±13.45
Production Yield	294.9±50.14	98.6±31.3	102.2±46.17
Sale Price	5.52±0.544	2.53±0.38	3.30±0.576
Cost of Total Plough	49.32±19.11	40.2±9.46	43.8±25.61
Cost of Total Sowing	113.9±31.80	72.7±8.69	129.1±54.19
Cost of Top Fertilizer	13.88±15.57	.	.
Cost of Disease and Pest Control	55.58±27.28	8.16±4.24	31.7±17.44
Cost of Harvest-Threshing	58.40±18.84	19.9±6.60	21.3±3.633
Cost of Carriage	8.04±6.533	24.5±8.86	9.92±12.71
Seed Quantity	1.17±0.391	1.15±0.40	1.25±0.498
Seed Variety	1.25±0.543	2.40±0.75	1.42±0.649
Production Risk Perception of The Producers	1.54±0.499	1.40±0.49	1.37±0.484
Profitability Share of The Producers	1.80±0.394	1.98±0.13	1.86±0.338
Farmer Experience	27.36±10.81	31.6±12.6	26.8±11.89
Agricultural Income	1.21±0.409	1.04±0.21	1.24±0.430
Membership to Agriculture Cooperatives/Chambers/Producer Unions	1.20±0.406	1.95±0.19	1.31±0.463
Information Source on Agricultural Issues	1.59±0.491	1.46±0.49	1.38±0.486
Agricultural Risk Perception	2.09±0.907	2.53±0.76	1.77±0.883
Technology	1.36±0.551	2.57±0.71	1.38±0.606
Adequate Equipment	1.39±0.558	2.05±0.77	1.40±0.592
Income	2.3±0.532	1.59±0.69	1.98±0.469

K-Means is one of the most prevalent algorithms in agricultural research due to its robust clustering capabilities. This algorithm is particularly effective for segmenting agricultural data into distinct groups based on similarities, thereby facilitating targeted analysis and decision-making. In the K-Means method, the cluster numbers are randomly selected at the initial stage. Reassignment of observations is performed by moving them to the nearest cluster based on their centroids. The K-means algorithm identifies k centroids for each cluster. The iterative process continues until the objective function based on squared error is minimized, that is, until each observation is assigned to clusters with the nearest centroid. The K-means method is a non-hierarchical method (Tatlidil 1996; Kuo et al., 2002; Alpar 2011; Akilli and Atil, 2020). In other words, the main purpose of the K Mean algorithm is to associate the nearest cluster center with the data points. Thus, a heterogeneous among themselves, homogeneous within itself data grouping is provided. In this study, In this study, Euclidean distance, which is commonly used to evaluate differences between data points in the K-Means clustering method, was employed as the measure of dissimilarity. Mathematical representation is given in Equation 1 for the objective function J (Orhan et al., 2011).

$$J = \sum_{i=1}^{K} (\sum_{k} \|x_k - c_i\|^2 \qquad [1]$$

where; K is the number of clusters, $c_i$is the centers of clusters, x_k is kth data point in ith cluster. Where $\|x_k - c_i\|^2$ is a distance measure between the centers of clusters c_i and kth data point x_k. With the implementation of the objective function, clusters are represented by the binary membership matrix U. The elements of matrix U are given in Equation 2 (Orhan et al., 2011).

$$u_{ij} = \begin{cases} 1 \ if \ \|x_k - c_i\|^2 \leq \|x_k - c_t\|^2, \forall t \neq i \\ 0 \qquad otherwise \end{cases} \qquad [2]$$

where u_{ij} shows that jth data point belongs to ith cluster, or not. Centroid of cluster c_i is given in Equation 3 (Ding and He, 2004; Faraoun and Boukelif, 2007; Orhan et al., 2011).

$$c_i = \frac{\sum_{j=1}^{N} u_{ij} x_j}{\sum_{j=1}^{N} u_{ij}} \qquad [3]$$

Multilayer perceptron

Neural networks (NN), a key technique within machine learning, are defined by their architecture of interconnected adaptive processing elements, which facilitate extensive parallel computations in the interpretation of high-dimensional data structures (Schalkoff, 1997). The utilization of artificial neural networks in the agricultural domain has seen a significant increase in recent years, demonstrating considerable success as an analytical tool in various agricultural applications. Advances in software and hardware technology play a positive role in the development of artificial neural networks day by day. In this process, significant progress has been made especially in the analysis of “big data” involving high feature dimensional data structures and data mining studies. Neural network classifiers are used successfully as an alternative to traditional methods in the classification of agricultural economics data. Classification has an important function in the realistic and objective evaluation of the existing structuring in agricultural systems. Structures formed by classification reveal the strengths and weaknesses of agricultural enterprises, whereby becoming an important source of information for decision-making units. In contrast to traditional statistical models, neural networks (NN) offer a comparatively novel methodology for developing classification models. Neural networks function as computing systems that emulate the human brain in their design and approach to information processing (Bharath and Drosen, 1993). It has a structure similar to the working principle of the human brain. As a computing system, Neural networks can accommodate multiple inputs and multiple outputs and may face two important situations in analysis. One of them is overfitting and the other is underfitting. If the model is too complex then overfitting may occur, and if the variables are too simple or insufficient, underfitting can be the result. In both cases, it reveals the restrictive effects of the generalization feature of neural networks (Duda et al., 2001).

In this study, a multilayer perceptron (MLP) neural network was employed. MLP networks are a type of model that performs supervised learning. The structure of an MLP consists of one input layer, one or more hidden layers, and one output layer (Liu et al., 2013). The neural network is provided with input data relevant to the research problem and corresponding outputs, initiating the training process. During this process, connection weights, the momentum coefficient, learning rate, and training algorithm are critical components. The architecture of the network design and the selection of training parameters must be determined prior to analysis. Optimal values for neural network parameters, such as the number of hidden layers and nodes per hidden layer, cannot be predetermined for a specific dataset and must be empirically identified by testing various parameter settings (Haykin, 1999; Marzban and Stumpf, 1996). The training algorithm's role is to adjust the connection weights. After completing the training process, the neural network is tested with new inputs, and evaluations are conducted by comparing the network outputs with the desired outputs. MLP networks utilize the generalized delta rule and the gradient descent algorithm.

The backpropagation algorithm which is one the supervised pattern recognition method, has two important steps: Propagation and update (Akıllı and Atıl, 2020). In the propagation phase: The data stream propagation occurs in a forward direction. Initially, weight and threshold values are assigned as random numbers. Once the weight sums of the neurons in the hidden layer are computed, they are processed through the activation function and transmitted to the output layer. During the backpropagation process, operations involve updating the weights, with errors associated with the output neurons being propagated backwards. This propagation and update cycle iteratively continues until the error criterion, typically measured as the sum of squared errors, reaches an acceptable level. In this paper, our minimization approach was determined as gradient descent optimization (commonly used with backpropagation learning algorithm) which was utilized in each network's learning process of the mean squared error at the output of the network. Net input (Net) is given in Equation 4 for NN. In Equation 4; the neuron number of input layer and hidden layer is expressed by k and j, respectively. Where, o_k

is the output of k layer and w_{kj} is the synaptic weight.

$$Net_j^a = \sum_{k=1}^{n} w_{kj} o_k \quad [4]$$

The output is expressed with sigmoid activation function as in Equation 5. β_j refers to the weight of the threshold element that connects to the neuron in the hidden layer (Öztemel, 2002; Akıllı, 2019).

$$o_j = \frac{1}{1 + e^{-(Net_j^a + \beta_j^a)}} \quad [5]$$

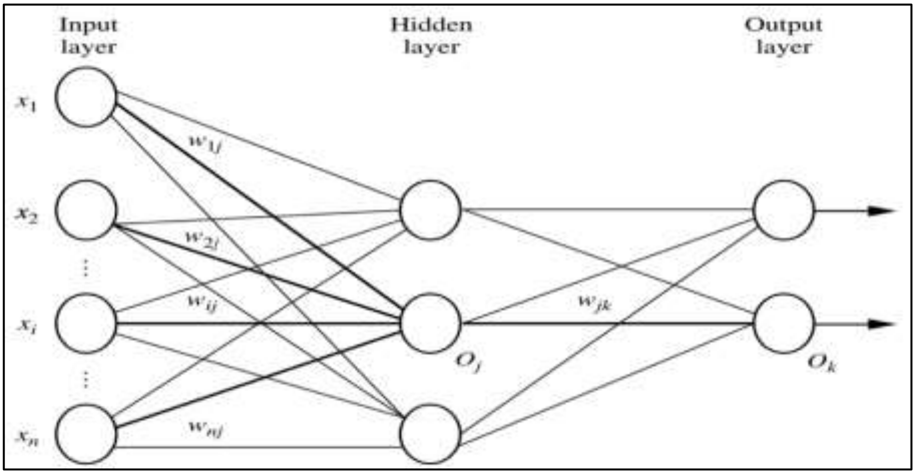

Figure 1. Multilayer perceptron (Han and Kamber, 2006).

Figure 1 illustrates the architecture of a multilayer perceptron (MLP). An MLP employing the backpropagation (BP) learning algorithm was developed, incorporating 1 to 3 hidden layers and 3 to 50 hidden neurons per layer. Various combinations of learning rates, momentum, and initial weights were tested to identify the optimal model parameters. These combinations were evaluated during the training, testing, and validation phases of the dataset. Tan-Sigmoid (Tan-Sig) and Log-Sigmoid (Log-Sig) activation functions were also assessed to compute the outputs from the weighted summation of neuron inputs in each hidden layer (Dongre et al., 2012). The maximum number of learning epochs was set to 10,000. All input parameters were normalized using the D-Min-Max normalization technique before being fed into the MLP network. The raw dataset was randomly divided into training (80%), testing (10%), and validation (10%) subsets to mitigate overfitting and underfitting issues. The network achieving the lowest mean squared error (MSE) on the test set was identified as the best-performing model. Accuracy metrics were evaluated at each phase of neural network modeling. In this study, different algorithms were utilized, running for up to 10,000 epochs or until convergence was achieved. Initial network weights and biases were randomly assigned. Table 3 provides a summary of the neural network architecture and training parameters used in this study.

Table 3. Summary of NN Structure

NN Structure	Descriptions
Model	Multilayer Perceptron
Connections	Feed-forward
Layer	1-3
Input Node	27-200
Hidden Node	3-35
Output Node	1
Activation Function	Tan-Sig, Log-Sig
Training Parameters	**Descriptions**
Mode	Supervised
Algorithms	Back-Propagation*
Weight Updates	Each Epoch
Learning Rate	0.01-0.03
Momentum Coefficient	0.80-0.95

**Bayesian Regularization (BR), Levenberg-Marquardt (LM), Scaled Conjugate Gradient (SCG), Conjugate Gradient Backpropagation with Powell-Beale Restarts (CGB), Brayde Fletcher Gold Farlo Shanno Quasi Newton Backpropagation (BFG).*

Performance assessment parameter of classifiers

The models' accuracy was evaluated by computing the mean squared error (MSE) classification accuracy (%) and Kappa statistics (Cohen, 1960). In the process of performance evaluation, the total accuracy was computed by using the test data for all types of producers. Statistical measures are given in Table 3. The equations of the error criteria used in this study are given in Table 4. The Kappa coefficient formula was given in Table 3 (Verma et al., 2020). Landis and Koch (1977) classified Kappa values into different categories: <0, 0–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80 and 0.81–1, which indicates slight, fair, moderate, substantial, and almost perfect fitting, respectively (Kuang et al., 2015).

Table 4. Statistical Error Criteria

Statistical Error Criteria	Equations
Mean Square Error (MSE)	$\mathrm{MSE} = \frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$
Accuracy %	$AC = \frac{TN+TP}{TN+FN+TP+FP}$
Kappa	$Kappa = \frac{N\sum_{i=1}^{r} X_{ii} - \sum_{i\_1}^{r}(X_{i+})(X_{i+})}{N^2 - \sum_{i=1}^{r}(X_{i+})(X_{i+})}$

Where for the i[th] *record,* $\hat{y}_i$*: predicted value,* y_i*: actual value, n: number of records.*
r: *Number of rows in the matrix,* X: *Number of observations in row i and column i (the diagonal elements),* x_{i+} *and* x_{ii} *are the marginal totals of row r and column i, respectively.* N: *Number of Examples.*

In the accuracy formula, TP represents the number of correctly predicted positive samples, known as true positives; FN denotes the number of positive samples incorrectly predicted as negative, referred to as false negatives; FP indicates the number of negative samples incorrectly predicted as positive, termed false positives; and TN represents the number of correctly predicted negative samples, referred to as true negatives (Orhan et al., 2011; Liu et al., 2013).

RESULTS AND DISCUSSIONS

A large number of NN architectures and K-Mean clustering algorithms were evaluated and tested in order to investigate the structural investigations of agricultural enterprises that produce dried bean, red lentil and chickpea in the Central Anatolia Region in the light of the knowledge of production, cost, labor and marketing strategies. The aim of our research is to explore the interrelationships between productive and financial variables and to find out how different types of farms are represented by the sample based on risk perception.

In Table 5-7, there are five different algorithms, two different activation functions and MSE performance values of hidden node combinations of various values of neural networks designed to classify according to risk perceptions of agricultural enterprises producing dry beans, red lentils and chickpeas, respectively. In the analysis, firstly, the number of hidden nodes was determined, and in the next process, the learning rates and momentum coefficients were changed in order to determine the most suitable values for the parameters, and operations were performed at optimal values. As can be seen in Table 5, from dried bean classification results, NN design of 27 input nodes, 10 hidden nodes, and output node with SCG algorithm and Tan-Sig activation function (MSE= 0.140) was chosen as 'optimal' for this data set. In other words, this optimal structure is the most successful system combination designed for classification of dried bean producers into high and low risk perception groups. In the results of the system established with the BR algorithm in Table 5, it is noteworthy that, unlike other algorithms, the BR algorithm only works with three and five hidden nodes. It is necessary to be very careful in determining the number of hidden nodes in high-dimensional data in terms of both the number of variables and the number of observations. In our study, we see the reflection of this situation as overfitting problem is encountered if analysis is made with a high number of hidden nodes in the BR algorithm.

Table 5. NN Classifier Results for Dried Bean Producers

Producer	Hidden Node Number	Back-Propagation Algorithms	MSE Training Log-Sig	MSE Training Tan-Sig	MSE Test Log-Sig	MSE Test Tan-Sig
Dried Bean	3	BR	0.034	0.038	0.212	0.150
	5		0.033	0.028	0.183	0.229
	5	LM	0.101	0.067	0.146	0.178
	10		0.074	0.050	0.160	0.143
	15		0.091	0.057	0.200	0.152
	5	SCG	0.111	0.096	0.151	0.150
	10		0.080	0.096	0.157	0.140
	15		0.087	0.094	0.167	0.165
	5	CGB	0.096	0.078	0.213	0.209
	10		0.080	0.088	0.182	0.191
	15		0.082	0.091	0.193	0.175
	5	BFG	0.113	0.107	0.145	0.164
	10		0.096	0.094	0.155	0.150
	15		0.106	0.081	0.175	0.188

Performance of neural network classifiers for red lentil producers are given in Table 6. Different from dried bean producers results, NN design of 27 input nodes, 5 hidden nodes, and output node with SCG algorithm and Log-Sig activation function (MSE= 0.100) was chosen as ‘optimal’ for this data set. In the red lentil classification results in Table 6, it has been observed that the BR algorithm works with different hidden node numbers compared to other algorithms, similar to dried bean applications. This situation is thought to be due to the structural features of the data set.

In classification performances of chickpea producers, as can be seen in Table 7, NN design of 27 input nodes, 15 hidden nodes, and output node with LM algorithm and Tan-Sig activation function (MSE= 0.119) was chosen as ‘optimal’. In the analysis process, neural network performances and the decisions regarding the optimal network structure were evaluated on the basis of test errors. In addition, training errors are included in our study as another element used in the evaluation of the training pattern of the network.

Table 6. NN Classifier Results for Red Lentil Producers

Producer	Hidden Node Number	Back-Propagation Algorithms	MSE			
			Training		Test	
			Log-Sig	Tan-Sig	Log-Sig	Tan-Sig
Red Lentil	3	BR	0.254	0.021	0.291	0.201
	5		0.024	0.024	0.164	0.179
	7		0.026	0.024	0.133	0.121
	5	LM	0.108	0.061	0.148	0.208
	10		0.084	0.057	0.196	0.145
	15		0.035	0.072	0.157	0.141
	5	SCG	0.054	0.056	0.100	0.156
	10		0.058	0.048	0.146	0.151
	15		0.060	0.214	0.135	0.239
	5	CGB	0.078	0.052	0.153	0.167
	10		0.062	0.060	0.132	0.137
	15		0.068	0.035	0.136	0.158
	5	BFG	0.072	0.068	0.127	0.155
	10		0.069	0.067	0.165	0.137
	15		0.056	0.097	0.143	0.196

Table 7. NN Classifier Results for Chickpea Producers

Producer	Hidden Node Number	Back-Propagation Algorithms	MSE			
			Training		Test	
			Log-Sig	Tan-Sig	Log-Sig	Tan-Sig
Chickpea	3	BR	0.050	0.049	0.167	0.163
	5		0.040	0.038	0.141	0.143
	7		0.039	0.022	0.256	0.167
	5	LM	0.114	0.131	0.142	0.135
	10		0.128	0.111	0.117	0.124
	15		0.092	0.104	0.154	0.119
	5	SCG	0.123	0.121	0.124	0.116
	10		0.126	0.132	0.128	0.112
	15		0.127	0.123	0.141	0.132
	5	CGB	0.129	0.122	0.121	0.114
	10		0.106	0.118	0.107	0.132
	15		0.126	0.104	0.117	0.154
	5	BFG	0.124	0.124	0.126	0.118
	10		0.133	0.124	0.131	0.103
	15		0.164	0.126	0.171	0.115

In general, it is seen that the training errors in the dried bean and red lentil results are calculated at lower values than the test errors. However, when the NN classifiers of chickpea producers were examined, it was determined that the training and test errors in SCG, CGB and BFG algorithms were very close to each other, or the training errors were larger than the test errors. This is thought to be due to the distribution of data structures or the presence of outliers. Figure 3 shows the MSE distributions of NN classifiers according to different algorithms and hidden node numbers on the basis of risk perceptions of pulses producers.

The classification accuracies of each model for both the NN and the K-Mean technique were also determined. In this context, the overall accuracy evaluations regarding the optimal results within the neural network designs configured for the pulses producer types are given in Table 8. The results show that; The overall accuracy obtained for dried bean, red lentil and chickpea producers was calculated as 68%, 81% and 72.5%, respectively.

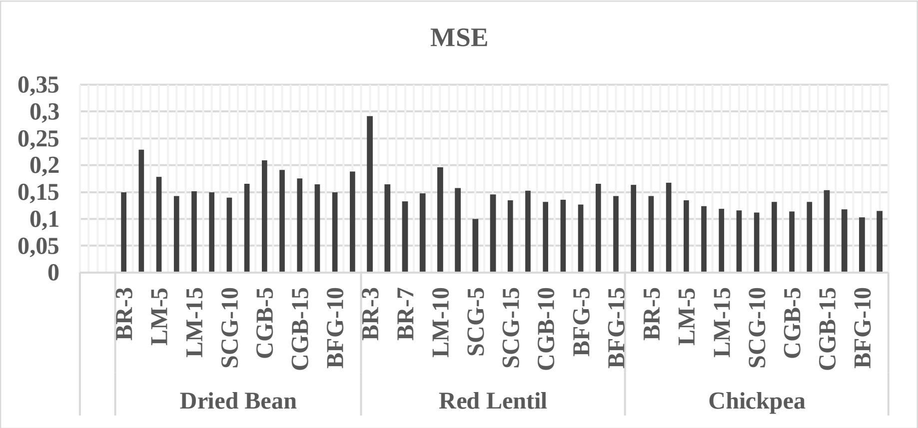

Figure 3. MSE for the different numbers of hidden nodes and back-propagation algorithm NN models.

In Table 8, K-Mean clustering technique results are given with the values of overall accuracy (%). The whole data set was used in clustering analyzes separately for three different producer types. As a result of the comparative evaluation of the results in Table 8 and Table 9, it is seen that neural networks have a more successful classification performance in all three producer types.

Table 8. Overall Accuracy for Optimal NNs

Producer	Cluster	Size	NN Model		Accuracy%
			Cluster1	Cluster2	
Dried Bean	Cluster1	18	10	8	68%
	Cluster2	32	8	24	
Red Lentil	Cluster1	40	36	4	81%
	Cluster2	30	9	21	
Chickpea	Cluster1	75	64	11	72.5%
	Cluster2	45	22	23	

Table 10 shows the overall accuracy (%), MSE, and Kappa coefficient for the classification algorithms used for pulses producers. The overall accuracy of 68%, 81%, and 72.5% and Kappa coefficient of 0.306, 0.613, and 0.383 of dried bean, red lentil, and chickpea showed the highest overall accuracy, respectively. Kappa-based assessments, MSE, and overall accuracy for each producer show that NN outperformed K-Mean classifications (Table 10). NN classifier provided higher accuracy than K-Mean technique for crop producers.

Table 9. Overall Accuracy (%) for K-Mean Technique

Producer	Cluster	Size	K-Mean		Accuracy%
			Cluster1	Cluster2	
Dried Bean	Cluster1	115	56	59	35.2%
	Cluster2	135	103	32	
Red Lentil	Cluster1	208	170	38	79.8%
	Cluster2	139	32	107	
Chickpea	Cluster1	374	171	203	47.1%
	Cluster2	226	114	112	

Table 10. Overall accuracy (%), MSE, and Kappa Coefficient for The Classification Algorithms

Producer	NN Model			K-Mean		
	Overall Accuracy%	MSE	Kappa Statistics	Overall Accuracy%	MSE	Kappa Statistics
Dried Bean	68%	0.140	0.306*	35.2%	0.648	-0.268*
Red Lentil	81%	0.100	0.613*	79.8%	0.201	0.583*
Chickpea	72.5%	0.199	0.383*	47.1%	0.528	-0.044

**Statistically significant ($p<0.05$)*

Table 11. Province Distribution of Classification Algorithms

Production Type	Province	NN Model*		K-Mean*	
		Cluster 1 (High Risk Perception)	Cluster 2 (Low Risk Perception)	Cluster 1 (High Risk Perception)	Cluster 2 (Low Risk Perception)
Dry Bean	Karaman	5(2)	0(3)	17 (30)	17(4)
	Konya	12(13)	12(11)	85 (129)	52(7)
	Nevşehir	1(3)	15(13)	43 (19)	16(40)
	Yozgat	0(0)	5(5)	14 (10)	7(11)
Red Lentil	Diyarbakır	15(15)	14(14)	58 (60)	47(45)
	Şanlıurfa	30(25)	11(16)	114 (140)	98(102)
Chickpea	Çorum	3(2)	3(4)	41 (36)	36(41)
	Karaman	25(27)	5(3)	22 (48)	45(19)
	Kırşehir	19(13)	7(13)	93 (81)	79(91)
	Konya	16(14)	13(15)	44 (44)	45(45)
	Nevşehir	3(2)	0(1)	14 (20)	18(12)
	Yozgat	20(17)	6(9)	71 (80)	92(83)

**Classified value (Observed value)*

Table 11 shows the distribution of high-risk perception (HRP) and low-risk perception (LRP) groups by provinces in all producer types. Accordingly, the results obtained according to the NN, and K-Mean clustering techniques and the observed values (in parentheses) based on the provinces where the research was carried out are summarized in Table 11. It has been observed that both methods have achieved very successful results both in general results and based on provinces. At the same time, Figure 4 shows the distribution of the results obtained by the K-Mean clustering technique according to the provinces where the research was conducted, on the basis of producers with high-risk perception (Cluster 1) and low risk perception (Cluster 2), and the observed values.

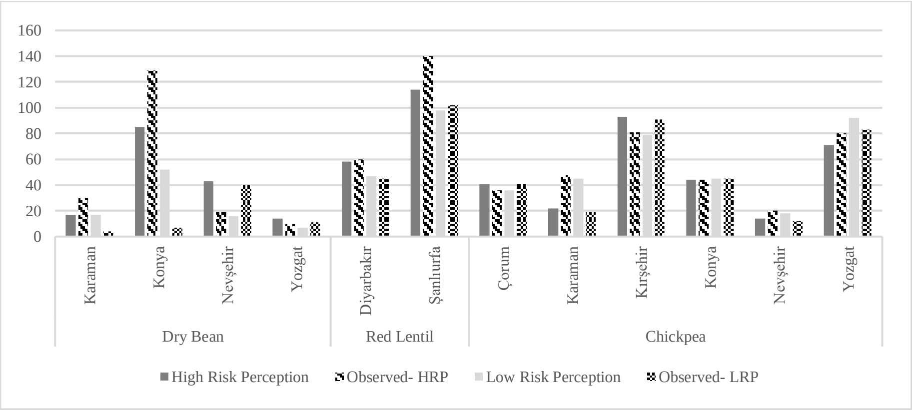

Figure 4. K-Mean clustering technique results based on province.

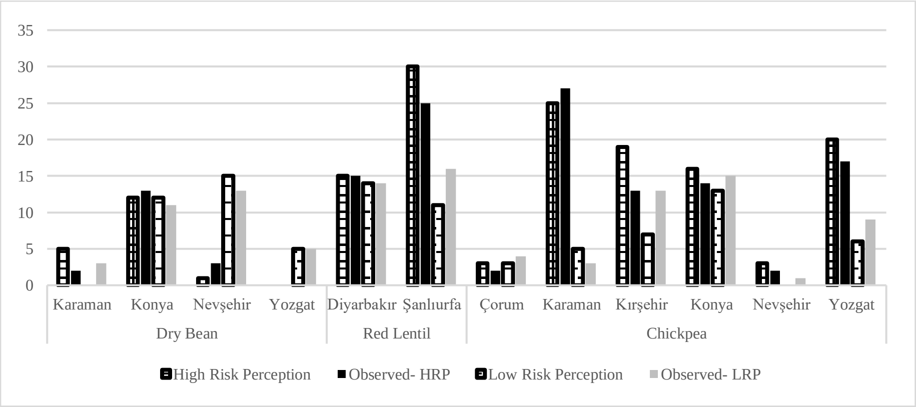

Figure 5. NN classifier results based on province.

Figure 5 shows the distribution of the results obtained by the NN classifier according to the provinces where the research was conducted, based on producers with high-risk perception (Cluster 1) and low risk perception (Cluster 2), and the observed values. As can be seen in the numerical values in Table 11 and in Figure 5, the results obtained from the NN classifiers, and the observed values are quite close to each other based on provinces. These successful results show that the training and test set determination processes are carried out appropriately in the construction of the neural network.

This study is one of the initial attempts to employ neural networks (NN) and K-Means clustering to classify crop producers. A review of various scientific investigations in economics and agricultural economics examines the utilization of classification functions, neural networks, and the K-Means clustering technique. Hruschka and Natter (1999) conducted a comparative analysis of a specifically designed feedforward artificial neural network, with a single hidden layer, and the K-Means clustering technique for addressing the issue of cluster-based market segmentation. Similar to our study, they compared NN and K-Means techniques and found that the feedforward neural network exhibited significantly lower squared-error values than K-Means across all segment numbers. Balakrishnan et al. (1996) examined the effectiveness of a particular neural network, the Frequency-Sensitive Competitive Learning Algorithm, in clustering data for strategic marketing decision-making. Unlike our study, their findings suggested that combining methodologies, where the outputs of the Frequency-Sensitive Competitive Learning network serve as initial seeds for K-Means, yielded segmentation schemes with greater managerial insights. Gallo et., (2013) applied an unsupervised artificial neural network to classify a series of olive farms. In our study, different from this study, we used the supervised NN model (MLP). Their study aims to identify and group companies with similar characteristics through a set of common indicators and create a rating for defining which companies are the best performing and how companies in the sector are related. Neural network performance was found to be positive, as in our study. Ghaffarian et al., (2022), considered together the concepts of risk and machine learning, similar to our study. They observed that production risk and impact/damage assessment are the most frequently addressed risk type and assessments that address risk components in machine learning-based farm risk management. Graskemper et al., (2021) analyzed the typologies of farmers based on a wide range of objective variables regarding their personal, farm, and context characteristics, which support an effective, target-group-specific design and communication of policies. Our study is quite similar to this study regarding the variables used. In this study, farmer typology was targeted, but in our study, producers were specifically classified according to their risk perceptions. Unlike our study, in this study, in which unsupervised neural networks were used, it is seen that the neural networks in our study obtained successful results. Jena and Majhi (2018) developed a novel artificial neural network (ANN) model to study farmers' behaviour towards decision-making on maize production in Kenya and they compared the accuracy level of functional link artificial neural network, multilayer perception, radial basis finction neural network models and discriminant analysis. Similar to our study, Jena and Majhi (2018)'s research considered different use of classification wherein inputs for crop production to classify farmer's behaviour. Examining three ANN classifiers, the authors indicated that the FLANN-based classification model provides the highest accuracy compared to the statistical model. This result seems to be compatible with the findings we obtained in our research, but unlike this study, we have examined MLP in more detail by using different algorithms and activation functions in our analysis. Kastens and Featherstone (1996) examined the accuracy of ordered multinomial logit models compared to feedforward backpropagation neural network models in predicting farmer risk preferences. Similar to our research, Featherstone (1996) explored the efficacy of backpropagation neural networks across different model structures. Additionally, the study encompassed an ordered multinomial logistic regression model within the statistical framework, and a naive model, which solely utilizes random draws from in-sample known responses without explanatory data, was examined for comparative purposes. The findings revealed that both ordered multinomial logistic regression models and neural networks possess the capability to predict a farmer's self-assessment of risk.

Moreover, it was noted that neural networks tend to surpass ordered multinomial logistic regression models in effectively distinguishing between individuals. Consistent with our study, neural networks demonstrated highly successful outcomes and provide several advantages to researchers over traditional statistical methodologies.

CONCLUSION

Machine learning approaches in farm management have recently been applied successfully with advances in technology and digitalization. In this study, it is aimed to classify the farmers into groups consisting of similar structures by using the socio-economic and technical characteristics of the farmers, based on the risky situation of the producers of pulses production. The NN classifier, which was developed within the framework of machine learning approach, was structured with different training algorithms and activation functions over the MLP model with a very detailed perspective, and applications were carried out on the data of pulse producers. It is essential to pay attention to the details of the neural network architecture while obtaining the optimal structure for the method studied, both in the preprocessing stage of the data and in the analysis process. The comparative classification results indicate that the NN results show a very successful performance compared to the K-Mean technique. The most successful training algorithms were determined as SCG for dried beans and red lentils, and LM algorithms for chickpeas, respectively. At the same time, the low error values obtained with the MLP network show that the NN method can be used successfully in modeling agricultural economics data.

It is thought that the distribution of risk groups by provinces will make important contributions to revealing the risky production potential and analyzing the farmer typology in agricultural policy design. It has been observed that most of the optimal architectures of neural networks have high explanatory power and very successful generalizability in the analysis of farmer typology. The simple and understandable architecture of the neural network method, the information available in the literature about the elements to be used in model construction, the computation speed, and the ability to work at very low error levels provide many advantages for the use of the neural network method. However, in addition to the advantages mentioned, there are also some limitations in the application of the method. Possible local extremes, high-dimensional data structures, noisy data, outliers, problems encountered in architectural design, and overfitting or underfitting problems can be given as examples of these limitations. In light of these results, future research might focus on identifying features from different Agri-sectors by integrating different machine learning techniques and considering alternative feature selection methods. Machine learning approaches offer a promising avenue. It is thought that our study will shed light on researchers who will work with different neural network models in the future.

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REFERENCES

Akilli A., Atıl H., 2020. Evaluation of normalization techniques on neural networks for the prediction of 305-day milk yield. Turkish Journal of Agricultural Engineering Research, 1(2): 354-367.

Akıllı A., 2019. Analysis Of Agricultural Data with Multivariate Nonlinear Fuzzy Regression Method. PhD Thesis, Kırşehir Ahi Evran University, Turkey.

Alpar R. 2011. Çok Değişkenli Istatistiksel Yöntemler. Detay Yayıncılık, 2011.

Andersen E., Elbersen B., Godeschalk F., Verhoog D., 2007. Farm management indicators and farm typologies as a basis for assessments in a changing policy environment. Journal of environmental management, 82(3): 353-362.

Bharath R., Drosen J., 1993. Neural Network Computing. Windcrest/McGraw-Hill, New York 188 pp.

Bozeman B., 2000. Technology transfer and public policy: a review of research and theory. Research Policy, 29(4-5): 627–655.

Chawgien K., Kiattisin S., 2021. Machine learning techniques for classifying the sweetness of watermelon using acoustic signal and image processing. Computers and Electronics in Agriculture, 181: 105938.

Dongre V.B., Gandhi R.S., Singh A., Ruhil A.P., 2012. Comparative efficiency of artificial neural networks and multiple linear regression analysis for prediction of first lactation 305-day milk yield in Sahiwal cattle. Livestock Science,147:192–197.

Duda R.O., Hart E.P., Stork G.D., 2001. Pattern Classification. John Wiley Sons, Inc., New York, USA.

Ellis F., 1993. Peasant Economics: Farm Households and Agrarian Development. 2nd edn. Cambridge University Press, Cambridge.

Erba M.E., Pratt J.E., Aplin R.D., Stephenson M.W., 1996. Comparison of costs and efficiencies between cooperative, proprietary, and captive fluid milk processors: A neural network approach. Work Paper of The Department of Agricultural, Resource and Managerial Economics, Cornell University.

Erba M.E., Pratt J.E., Aplin R.D., Stephenson M.W., 1996. Comparison of costs and efficiencies between cooperative, proprietary, and captive fluid milk processors: A neural network approach. Work paper of the department of agricultural. Resource and Managerial Economics, Cornell University.

Gallo C., Contò F., La Sala P., Antonazzo A.P., 2013. A neural network model for classifying olive farms. Procedia Technology, 8, 593-599.

Ganpat, W., Bekele, I. 2001. Looking for the trees in the forest: farm typology as a useful tool in defining targets for extension. In Emerging Trends in Agricultural and Extension Education (JR Lindner, ed.), Proceedings of the 17th Annual Conference of the Association for International Agricultural and Extension Education (pp. 4-7).

Ghaffarian S., van der Voort M., Valente J., Tekinerdogan B., de Mey Y., 2022. Machine learning-based farm risk management: A systematic mapping review. Computers and Electronics in Agriculture, 192, 106631.

Gopinath M., Batarseh F.A., Beckman J., Kulkarni A., Jeong S., 2021. International agricultural trade forecasting using machine learning. Data Policy, 3: 1-20.

Goswami R., Chatterjee S., Prasad B., 2014. Farm types and their economic characterization in complex agro-ecosystems for informed extension intervention: Study from coastal West Bengal, India. Agricultural and Food Economics, 2: 1-24.

Graskemper V., Yu X., Feil J.H., 2021. Farmer typology and implications for policy design–An unsupervised machine learning approach. Land Use Policy, 103: 105328.

Han J., Kamber M., 2006. Data Mining: Concepts and Techniques. 2nd ed.;

Elsevier: San Francisco, USA.

Haykin S., 1999. Neural Networks: A Comprehensive Foundation. 2nd Ed. Prentice-Hall, Upper Saddle River, Nj.

Hruschka H., Natter M., 1999. Comparing performance of feedforward neural nets and K-means for cluster-based market segmentation. European Journal of Operational Research, 114(2): 346-353.

Jena P.R., Majhi R., 2021. An application of artificial neural network classifier to analyze the behavioral traits of smallholder farmers in Kenya. Evolutionary Intelligence, 14(2): 281-291.

Joerding W.H., Li Y., Young D.L., 1994. Feedforward neural network estimation of a crop yield response function. Journal of Agricultural and applied Economics, 26: 252-63.

Kastens L.T., Featherstonem A.M., 1996. Feedforward backpropagation neural networks in prediction of farmer risk preferences. American Journal of Agricultural Economics, 78: 400-415.

Kohzadi N., Boyd M.S., Kermanshahi B., Kaastra I., 1996. A comparison of artificial neural network and time series models for forecasting commodity prices. Neurocomputing, 10(2): 169– 181.

Koklu M., Ozkan I.A., 2020. Multiclass classification of dry beans using computer vision and machine learning techniques. Computers and Electronics in Agriculture, 174: 105507.

Kostrowicki J., 1977. Agricultural typology concept and method. Agricultural Systems, 2(1): 33–45.

Kung H.Y., Kuo T.H., Chen C.H., Tsai P.Y., 2016. Accuracy analysis mechanism for agriculture data using the ensemble neural network method. Sustainability, 8(8): 735.

Kuo R.J., Ho L. M., Hu C.M., 2002. Cluster analysis in industrial market segmentation through artificial neural network. Computers Industrial Engineering, 42: 391-399.

Liu M., Wang M., Wang J., Li D., 2013. Comparison of random forest, support vector machine and back propagation neural network for electronic tongue data classification: Application to the recognition of orange beverage and Chinese vinegar. Sensors and Actuators B: Chemical, 177: 970-980.

Mahapatra A.K., Mitchell C.P., 2001. Classifying tree planters and non planters in a subsistence farming system using a discriminant analytical approach. Agroforestry Systems, 52(1):41–52.

Maponya M.G., Van Niekerk A., Mashimbye Z.E., 2020. Pre-harvest classification of crop types using a Sentinel-2 time-series and machine learning. Computers And Electronics in Agriculture, 169: 105164.

Marzban C., Stumpf G., 1996. A neural network for tornado prediction based on Doppler radar- derived attributes. Journal of Applied Meteorology and Climatology, 35: 617–626.

Orhan U., Hekim M. Ozer M., 2011. EEG signals classification using the K-means clustering and a multilayer perceptron neural network model. Expert Systems with Applications, 38: 13475-13481.

Oztemel E., 2002. Yapay sinir ağları. Papatya Yayıncılık: İstanbul, Turkey.

Richards T.J., Patterson P.M., Van Ispelen P., 1998. Modeling fresh tomato marketing margins: econometrics and neural networks. Agricultural and Resource Economics Review, 27(2), 186-199.

Schalkoff R. J., 1997. Artificial Neural Network. New York: McGraw- Hill.

Shortridge J.E., Guikema S.D., Zaitchik B.F., 2016. Machine learning methods for empirical streamflow simulation: a comparison of model accuracy, interpretability, and uncertainty in seasonal watersheds. Hydrology and Earth System Sciences, 20: 2611–2628.

Soule M.J., 2001. Soil management and the farm typology: Do small family farms manage soil and nutrient resources differently than large family farms? Agricultural and Resource Economics Review, 30:179–188

Stastny J., Turcinek P., Motycka A., 2011. Using neural networks for marketing research data classification. Mathematical Methods and Techniques in Engineering and Environmental Science, 5: 253-256.

Tatlidil H., 1996. Uygulamalı Çok Değişkenli Istatistiksel Analiz. Cem web Ofset, Ankara.

Timothy W.O., 1994 Identifying target groups for livestock improvement research: The classification of sedentary livestock producers in western Niger. Agricultural Systems, 46: 227–237.

Tongcham P., Supa P., Pornwongthong P., Prasitmeeboon P., 2020. Mushroom spawn quality classification with machine learning. Computers and Electronics in Agriculture, 179: 1-8, 105865.

Trenz O., Šťastný J., Konečný V., 2011. Agricultural data prediction by means of neural network. Agricultural Economics, 57(7): 356-361.

Vanclay J.K., 2005. Using a typology of tree-growers to guide forestry extension. Annals of Tropical Research, 27: 97–103.

Viana C.M., Santos M., Freire D., Abrantes P., Rocha J., 2021. Evaluation of the factors explaining the use of agricultural land: A machine learning and model-agnostic approach. Ecological Indicators, 131: 108200.

CHAPTER 12

BOOTSTRAP VS. POSTERIOR PROBABILITY: THE BEST METHOD FOR PLANT PHYLOGENY

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INTRODUCTION

Over the past ten years or more, phylogenetic research has shifted its primary focus (Erixon et al, 2003). Utilising the information contained in their inheritable properties, such as DNA sequences or morphology, phylogenetic analysis aims to establish the historical links between entities, such as genes or animals. In order to illustrate the relationships within the data, these inferences depend on a particular statistical model. In 2003, Cumbis et al. Measuring the amount of support for particular groups of species has replaced the objective of finding the most correct arrangement of species according to a specified criterion (Erixon et al., 2003). In addition to drawing conclusions, many studies try to assess the validity of these conclusions by calculating a statistic that can be interpreted according to reliability, robustness, confidence, or support. Since it gives a numerical evaluation of the combined strength of the data and the chosen model, quantifying the level of support for inferences made in phylogenetic reconstruction is vital (Cummings, et al 2003).

A few often-used metrics include Bootstrap values, Bremer support values, jackknife values, and Bayesian posterior probabilities. These were first proposed by Felsenstein (1985), Bremer (1988, 1994), Farris et al. (1996), Yang and Rannala (1997), and Huelsenbeck et al. (2001). Cases where the meaning of support is not always related to probability include Bremer support, certain bootstrapping and jackknifing interpretations, and others (Farris et al., 1996; Oxelman et al., 1999). There are writers who have favoured the use of a probabilistic measure of support above others (Felsenstein, 1985; Sanderson, 1989, for example). Based on the facts and assumptions, this metric determines the chance that a certain clade is true. The probabilistic descriptions of bootstrapping and Bayesian inference will now occupy the whole of this article. Phylogenetic methods need to be robust, consistent, efficient, and falsifiable (Penny et al., 1992). It follows that methods that obtain high support values for correct clades with less data are preferable in terms of support. Zharkikh and Li (1992) and Hillis and Bull (1993) are among the writers who argue that nonparametric bootstrapping is conservative. While Ellison et al. (2003) found that bootstrap frequencies do provide unbiased probability estimates in a Bayesian setting, Efron et al. (1996) contended that bootstrap frequencies are not inherently biassed downwards. This review set out to do just that—evaluate and characterise the relationship between the posterior probability and the fraction of bootstrap replicates—two popular statistical metrics in phylogenetics.

The Bootstrap

Efron (1979) and Efron and Tibshirani (1993) describe the nonparametric bootstrap, a statistical method that uses data resampling to estimate values of interest. Felsenstein was the first to use it in a phylogenetic study (1985). The bootstrap method is used in phylogenetic research to select characters (like nucleotide sites) at random from the original data. It is possible to select the same character many times. After that, a new bootstrap sample with the same size as the old one is generated using these chosen characters. After that, we can use the bootstrap sample to infer a phylogenetic tree using neighbour joining, maximum parsimony, or maximum likelihood, among other methods. Following a predetermined number of repetitions, the process is consolidated (Cummings et al., 2003). A common tool in phylogenetic analysis, the bootstrap provides a measure of "confidence" or "support" for the inferred phylogenetic relationships from the data and helps to estimate the reliability of tree topology. Furthermore, it is still the gold standard when it comes to assessing inferred phylogenetic relationships, particularly in the plant kingdom.

The technique is based on the notion of recurrent sampling from observable data and follows a frequentist perspective. Offers estimations of the level of uncertainty in parameter estimations by taking into account the variability noticed in the collected data. This method is often used when the assumptions of parametric models, such as normality, are plausible and when computing feasibility is a consideration.

The Posterior Probabilities

For phylogenetic tree-based inferences, Bayesian analysis uses posterior probability (Erixon et al., 2003). According to several studies (Rannala and Yang, 1996; Yang and Rannala, 1997; Larget and Simon, 1999; Mau et al., 1999; Newton et al., 1999; Cummings et al., 2003), Bayesian methods have greatly improved phylogenetic analysis, particularly when coupled with Markov chain Monte Carlo (MCMC) methods. The prior probability and the likelihood of the data given a specific model determine the posterior probability, according to Bayes' theorem. This equation has not seen much use until recently since it cannot be solved analytically (Erixon et al., 2003). The

Bayesian approach may determine the interest variable's posterior probability given a prior probability, a likelihood function, and some data. The hard nature of directly calculating computing posterior probabilities has prompted the usage of stochastic estimating methods like MCMC. (Yang and Rannala, 1997; Cummings et al, 2003) Phylogenetic tree topology posterior probabilities can be estimated using Markov Chain Monte Carlo (MCMC) techniques. Using explicit models of evolution, these techniques enable support studies to be performed on data sets with hundreds of species (Huelsenbeck et al., 2001; Erixon et al., 2003). Once the process approaches stationarity, the posterior probability can be correctly determined by counting the number of observations of each parameter value (Cummings et al., 2003).

The Bayesian approach involves combining observed data with past assumptions to update perspectives on parameters or hypotheses. Provides a posterior distribution instead of a single estimate, which includes uncertainty in a more comprehensive manner than frequentist confidence intervals. Particularly beneficial in scenarios when prior information is accessible or when dealing with complex models where assumptions about data distribution are not apparent.

Bootstrap vs Posterior Probability

In phylogenetic analysis, the exact relationship between bootstrap results and posterior probability values is a major and unsolved problem. Efron et al. (1996) argue that the theories supporting the two metrics should be considered equivalent, despite the fact that they are mostly independent. Contrary to popular belief, these statistics can differ according to a number of studies (e.g., Buckley et al., 2002; Leach'e and Reeder, 2002; Suzuki et al., 2002; Whittingham et al., 2002; Wilcox et al., 2002; Alfaro et al., 2003; Douady et al., 2003). That is why additional studies comparing and contrasting the bootstrap and posterior probability metrics are necessary. A clear analytical answer is unfortunately not readily apparent (Cummings et al., 2003).

In the process of contrasting two metadologies on the basis of their respective philosophies, we can conclude that the bootstrap is founded on frequentist statistics and places an emphasis on the variability of estimates that is caused by sampling. Bayesian statistics, on the other hand, are the foundation of posterior probability. This kind of probability takes into account previous beliefs and then updates them depending on the evidence that has been seen. Furthermore, while we are comparing the two methods based on their respective outputs, we may get to the conclusion that the bootstrap method often offers confidence intervals or ranges around point values, such as averages or regression coefficients. It is possible to generate a variety of summaries, such as credible intervals, from the posterior distribution that is provided by posterior probability that is provided (Zander, 2004).

Last but not least, with regard to their applications, we can mention that the bootstrap is often used in situations where direct parameter estimate from data is required, supposing that the data adheres to certain parametric assumptions. For the purpose of integrating previous information or rigorously assessing uncertainty, posterior probability is used, particularly in the context of complicated modelling situations (Nylander et al., 2008)

In summary, while both bootstrap and posterior probability aim to quantify uncertainty, they do so from different philosophical and methodological standpoints, catering to different types of statistical problems and data analysis approaches.

What About Plant Phylogenetic Studies?

In plant phylogenetics both bootstrap support values and posterior probability are used to evaluate the dependability of inferred phylogenetic trees. Nevertheless, they stem from distinct statistical methodologies and possess divergent explanations.

Bootstrap support quantifies the degree to which a resampled data-based phylogenetic tree may be trusted. It comprises replacing a small subset of the original data with a larger subset at random to create several pseudoreplicate datasets. Hence, for every pseudoreplicate dataset, phylogenetic trees are built and the percentage of trees that include a certain clade or branch is determined. An empirical estimate of the frequency of a given clade in the trees obtained from the resampled data is therefore provided. More certainty in the presence of the clade is indicated by higher scores, which might range from 0% to 100%. Nevertheless, there are drawbacks to every method; for example, this one needs a lot of trees to generate and could not show the real likelihood of a clade's existence since it doesn't take model uncertainty into consideration.

The posterior probability is a Bayesian statistic that determines the likelihood of a phylogenetic hypothesis being true in light of the evidence that has been seen and the prior distribution. In other words, it is a representation of the percentage of trees that were sampled from the posterior distribution and include a certain clan. In the approach, it is used in Bayesian inference methods, which commonly make use of Markov Chain Monte Carlo (MCMC) procedures. An estimation of the posterior distribution is obtained via the study by integrating across all of the available trees and model parameters. In addition, the values of the posterior probability range from 0 to 1 (or 0% to 100%), which represents the probability that a clade is correct given the data and the model. A posterior probability of 1 indicates absolute certainty (given the model and the data) in the existence of the clade, whereas a value that is close to 0 indicates that there is no support for the existence of the clade. Nevertheless, much like any other method, it has limitations, such as being dependent on the prior distributions that are selected, which might have an effect on the outcomes. On top of that, particularly for complicated models and extensive datasets, it is necessary. Additionally, if the priors are not carefully selected or if the model is extremely basic, it have the potential to be unduly confident (Huelsenbeck and Ronquıst. 2001).

The primary distinction lies in the fact that bootstrap is a technique rooted in frequentist statistics, while posterior probability is a method based on Bayesian statistics. Additionally, the bootstrap values are derived via resampling the data, whereas the posterior probabilities take into account past information and include the full parameter space. Furthermore, posterior probabilities often surpass bootstrap values since they represent the likelihood of a clade based on the data and model, rather than merely the occurrence frequency in resampled datasets. Finally, both approaches have their own applications, and researchers often use both to provide a more thorough overview of the evidence supporting their evolutionary ideas.

Despite the fact that we can compare the two methods on a wide variety of features, in terms of their practical application to plant phylogenetics, we can state that while studies using maximum likelihood or parsimony methods typically use bootstrap, Bayesian phylogenetic analyses, which incorporate model uncertainty and prior information, use posterior probability.

Phylogenetic studies often present both values to give a more solid evaluation of clade support (Zhaxybayeva and Gogarten, 2002)

Example Studies For Using Techniques in Plant Phylogenetics

Karaman Erkul et al. (2022) studied on a new *Astragalus* L. species. They used ITS gene region and they analysed the sequences by using MEGA program for bootstrapping method. In the study phylogenetic tree inferred by maximum likelihood with the GTR model additionally with bootstrap method which was burned under 1000. The values were indicated near the branches. According to the values the new species was defined (Fig 1).

Also, Aytaç et al.(2022) worked on Dianthus L. species. They reported a new species *Dianthus berkayii* based on both morphological features and molecular data. They used both bootstrap analysis with 1000 replicates and posterior probability value of over 1 with 10000000 MCMC runs. According to the values on the tree they reported that it was a new species (Figure2).

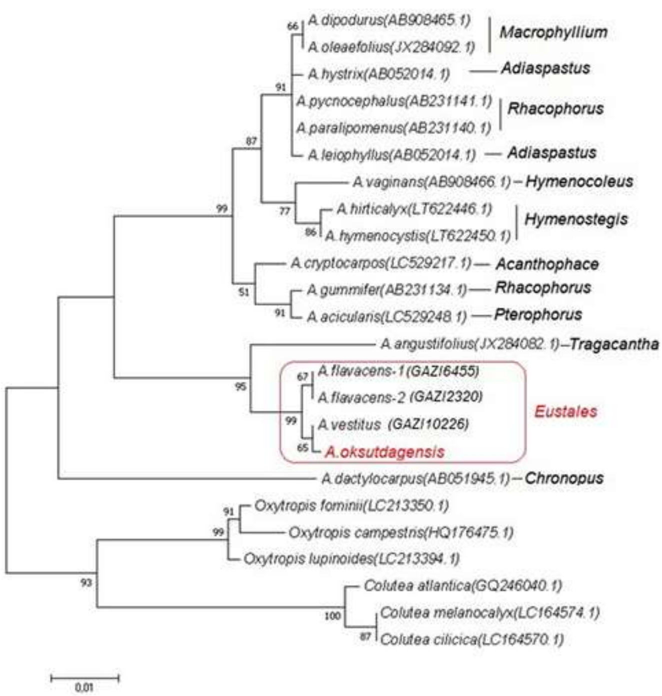

Figure 1: Karaman Erkul et al. (2022)'s study by using bootstrapping values in ML analysis.

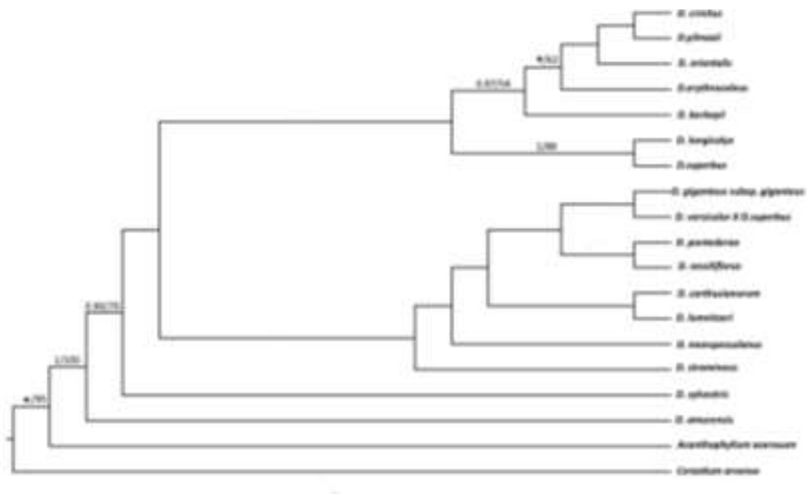

Figure 2: Aytaç et al. (2022)'s study by using both bootstrapping and posterior probability values in ML analysis.

Another study was done by Şahin et al.(2024) a new species from *Dorycnium* genus. New species *Dorycnium vuralii* was defined based on both pollen, morphological features and molecular data. Both bootstraping values and posterior probability values were analysed with 1000 replicates and 10000000 MCMC runs. Both values were written on the phylogenetic tree to indicate the separations (Figure 3).

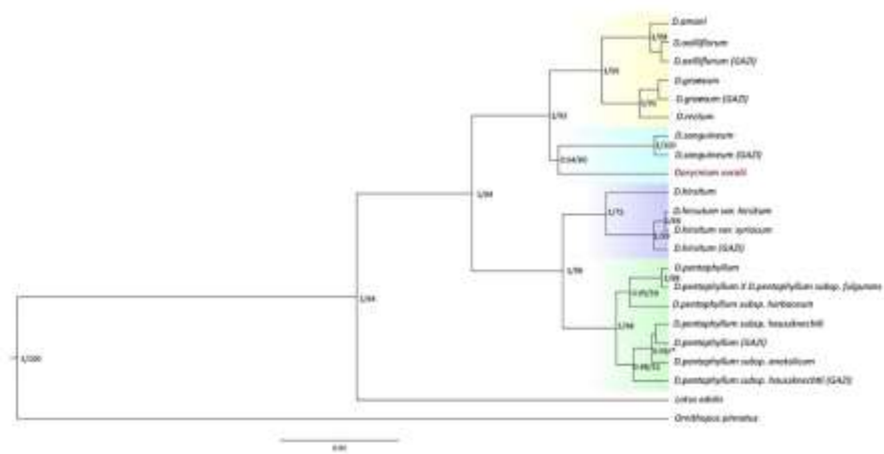

Figure 3: Bayesian consensus tree with ML likelihood parameters additionally with both bootstraping method and posterior probability method (Şahin et al.,2024)

CONCLUSION

Any phylogenetic hypothesis will always run the danger of being disproved in light of fresh evidence. This is due to the fact that discovering the real connection between taxa is extremely uncommon, assuming it is ever possible to do so at all. Positive support values are not sufficient to ensure proper conclusions; only conclusions that are adequately supported may do so. When more data is collected from the same phylogeny, the conclusions drawn from that data are more trustworthy and robust. Therefore firstly more data gives more realible results. Secondly, more technique gives more accurate results. And thirdly combination of more technique and data gives the best results in phylogenetic studies.

After the invention of these statistical texhniques, the scientists understand that for analysing the best combination of these techniques will give the best. Then in many studies they combine all these analysis together for accurate results. Especially, plant phylogenetic studies due to the maternally inheriting fetatures of the organells, the combination of different regions of cpDNA and nrDNA data with many techniques gives the best results.

In conclusion both bootstrap support and posterior probability are crucial methods in plant phylogenetics, each providing distinct advantages and valuable insights. Utilising both methods together may provide a more intricate and all-encompassing comprehension of the evolutionary connections between plant species.

REFERENCES

Alfaro, M. E., S. Zoller, And F. Lutzonı. 2003. Bayes or bootstrap? Aaimulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Mol. Biol. Evol*. 20:255–266.

Aytaç, Z., Kandemir, A., Ertuğrul, T., Fişne, A., & Ateş, M. A. (2022). Dianthus berkayii (Caryophyllaceae), a new species from Turkey. *Turkish Journal of Botany*, *46*(3), 260-270.

Bremer, K. 1994. Branch support and tree stability. *Cladistics* 10:295– 304.

Bremer,K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42:795–803.

Cummıngs,M. P., S. A.Handley, D. S. Myers, D. L. Reed, A. Rokas, And K.Wınka. 2003. Comparing bootstrap and posterior probability values in the four-taxon case. *Syst. Biol*. 52:477–487.

Douady, C. J., F. Delsuc, Y. Boucher, W. F. Doolıttle, And E. J. P. Douzery. 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability.*Mol. Biol. Evol.* 20:248–254.

Efron, B., E. Halloran, And S. Holmes. 1996. Bootstrap confidence levels for phylogenetic trees. *Proc. Natl. Acad. Sci*. USA 93:7085– 7090.

Erixon, P., Svennblad, B., Britton, T., & Oxelman, B. (2003). Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic biology*, *52*(5), 665-673.

Farrıs, J. S., V. A. Albert, M. K¨ Allersj¨O , D. Lıpscomb, And A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12:99–124.

Felsensteın, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791.

Hıllıs, D. M., And J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol*. 42:182–192.

Huelsenbeck, J. P., And F. Ronquıst. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17:754–755.

Karaman Erkul, S., Duman, H., & Ateş, M. A. (2022). Astragalus oksutdagensis (Fabaceae), a new species from Turkey. *Nordic Journal of Botany, 2022*(3).

Larget, B., And D. L. Sımon. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Mol. Biol. Evol*. 16:750–759.

Leach´E, A. D., And T. W. Reeder. 2002. Molecular s ystematics of the eastern fence lizard*Sceloporus undulatus*:Acomparison of parsimony, likelihood, and Bayesian approaches. *Syst. Biol*. 51:44–68.

Morrison, D. A. (2010). Bayesian posterior probabilities: revisited. *arXiv preprint arXiv:1001.3638*.

Nylander, J.A.A., J.C. Wilgenbusch, D. L. Warren, and D. L. Swofford. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24(4): 581-583.

Oxelman, B., M. Backlund, And B. Bremer. 1999. Relationships of the Buddlejaceae s.l. investigated using parsimony jackknife and branch support analysis of chloroplast *ndh*F and *rbc*L sequence data. *Syst. Bot*. 24:164–182.

Penny, D., M. D. Hendy, And M. A. Steel. 1992. Progress with methodsbfor constructing evolutionary trees. *Trends Ecol. Evol*. 7:73–79.

Şahin, B., Karaman, S., Ateş, M. A., & Aytac, Z. (2024). Dorycnium vuralii (Fabaceae), a new species from Türkiye. Turkish Journal of Botany, 48(2), 120-134.

SandersoN, M. J. 1989. Confidence limits on phylogenies: The bootstrapbrevisited. *Cladistics* 5:113–129.

Suzukı, Y., G. V. Glazko, And M.Neı. 2002. Overcredibility of molecular phylogenetics obtained by Bayesian phylogenetics. Proc. *Natl. Acad. Sci*. USA 99:16138–16143.

Whıttıngham, L. A., B. Slıkas, D. W. Wınkler, And F. H. Sheldon. 2002. Phylogeny of the tree swallow genus *Tachycineta* (Aves: Hirundinidae), by Bayesian analysis of mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* 22:430–441.

Yang, Z., And B. Rannala. 1997. Bayesian phylogenetic inference using DNA sequences: A Markov chain Monte Carlo method. *Mol. Biol. Evol.* 14:717–724.

Zander, R. H. (2004). Minimal values for reliability of bootstrap and jackknife proportions, decay index, and Bayesian posterior probability. *Phyloinformatics*, *2*, 1-13.

Zharkıkh, A., And W.-H. Lı. 1992. Statistical properties of bootstrap estimation of phylogenetic variability from nucleotide sequences. I. Four taxa with a molecular clock. *Mol. Biol. Evol.* 9:1119–1147.

Zhaxybayeva, O., & Gogarten, J. P. (2002). Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC genomics*, *3*, 1-15.

CHAPTER 13

FATE AND BEHAVIOR OF HEAVY METALS IN SOIL SYSTEMS AND THEIR RISK ASSESSMENT

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INTRODUCTION

Soil and water sources are getting polluted day by day due to many anthropological causes. Unconscious agricultural applications such as excessive use of fertilizers not only decrease the fertility of soils, but also contaminate them with various metals they contain. Exhaust gasses from vehicles are also sources for some heavy metals which first pollute air, then soil since the particles end up on soil either by settling by gravity or by meteorological precipitations. Industrial wastes possess a serious problem in terms of heavy metal contamination to both soil and water sources. Waste effluents of various industries as well as municipality waste waters contain serious amounts of different heavy metals which end up in soil and water bodies.

Besides the human factor, presence of mines is already a cause of soil contamination. Yet the attempt to dig and extract the mines are a bigger source for soil contamination with the relevant mines. Volcanic eruptions may also contaminate the soil in various parts of the world.

Soil is a crucial component of all ecosystems. Soil health determines the health of agricultural crops, animal health and-directly and indirectly- human health. This way or the other, soils of earth, especially the agricultural lands are under constant attack of heavy metal pollution. Identifying the heavy metals, their contamination sources, their fate in soil is the first step towards their remediation from soil. Moreover, the risks of heavy metal contaminations for the human health and environment should be well addressed.

Heavy metals are the elements with high atomic weight. They possess toxic effects in low or high concentrations in environment. Metals such as iron, copper, manganese molybdenum, zinc, selenium, nickel and chromium take roles in biochemical reactions in organisms' bodies. They are also referred as trace elements and are needed in low amounts are toxic in high concentrations. On the other hand, metal elements such as arsenic, aluminum, mercury, lead uranium, antimony, barium, platinum, etc. have no known vital biological actions and thus called as non-essential elements (Tchounwou et al., 2012). All these elements are dispersed in the environment due to several human activities and possess toxicity and therefore referred to as contaminants, or pollutants, especially above certain thresholds. Heavy metals enter and contaminate environment either by natural or anthropological sources. Natural causes are volcanic eruptions, rock weathering or atmospheric depositions. Industries and their wastes, traffic, sewage, mining etc. are among the human causes of heavy metal contamination to environment (Kanwar et al., 2020). In human body, the presence of heavy metals, which may enter via the consumption of fruits, vegetables, and other agricultural crops grown on soils with heavy metal pollution, can cause the formation of tumors since they can affect DNA, organelles and enzymes taking place in biochemical reactions (S. Wang & Shi, 2001). Heavy metals damage DNA synthesis in plants besides harm to chlorophyll synthesis, enzymes and hence the metabolism, and also hinder growth. As a result, low and unhealthy yields are obtained. Heavy metals in soil are the first key to evaluate soil heath and therefore agricultural crop, animal and human health effects of them. This chapter will mention about the heavy metal contaminations in soil and their risk assessments.

Aluminum

Aluminum (Al) is an element of atomic number 13. It is widely used in industries and it is almost impossible to avoid in today's world. It is used in a wide spectrum from construction to food packaging, from automotive industry to kitchen utensils. Moreover, they are utilized in some medications such as antacids, deodorants and food additives. It is also important for wastewater treatment for the reduction of organic matter (WHO, 2017a). A minimal risk level of 1 mg/kg a day was determined to be toxic in long term usage (Agency for Toxic Substances & and Disease Registry, 2011). The toxic form for human health is the free metal cation Al3+ form (Exley, 2017). Some tissues are especially selected for the accumulation of aluminum in human body. One and most important of these tissues is brain. Therefore, accumulation of this heavy metal is thought to be related to Alzheimer's disease, multiple sclerosis and autism (Exley & Clarkson, 2020). Apart from direct exposure via usage of utensils or deodorants, consumption of plants with aluminum accumulated can be an underestimated source of exposure, especially in terms of long-term exposure and accumulation.

When contaminate soil from various sources, aluminum can become toxic to plants, especially in acidic soils. Today almost half of the agricultural soils are contaminated with Al all over the world. When in low concentration, Al can enhance nutrient uptake, root and shoot growth of plants, therefore can be beneficial. However, when the dosage rises, this heavy metal inhibits cell division, lipid peroxidation, root growth, water and nutrient uptake, ATP synthesis and also harms DNA, and hence becomes toxic to plants (Bojórquez-Quintal et al., 2017; Panda et al., 2009). Via plant consumption, Al can enter human body. An example is tea, *Camellia sinensis*, whose infusion drink leads to Al transport to body when grown on Al contaminated soil (Ashenef, 2014; Mehra & Baker, 2007).

In order to clean up aluminum contamination from soils, bio and phytoremediation techniques have been developed. In bioremediation, microorganisms are utilized and the toxic compounds are either converted into a non-toxic or less toxic form. In phytoremediation, plants of many different species are grown on contaminated soil and the concentration of of pollutant is lowered either by stabilization in the roots or around the root area, or transportation of the pollutants to the plant body and tissues. The plant with accumulated pollutant can then be harvested and processed. Three plant species were studies for their aluminum accumulation capacities. These plants are intensely consumed as food. *Raphanus sativus* (radish) *Vigna radiata* (mung bean) and *Cicer arietinum* (chickpea) were shown to accumulate the heavy metal aluminum (Raj & Rebecca, 2014). Therefore, the consumption of such plants grown on aluminum contaminated soil might possess a problem for human health in long term. Moreover, they can be used to clean up heavy metals from the soil. The performance of the remediation techniques can also be enhanced by using both of them together. For instance, cowpea used to test the aluminum accumulation from soil and use of a mycorrhizal fungi was suggested to enhance the phytoremediation of Al by cowpea (Alori & Fawole, 2012). *Scirpus grossus* and *Thypa angustifolia* were shown to be effective on removal of Al from soil. They were reported to tolerate up to 500 mg/kg Al concentration. When a bacterial species (*Vibrio alginolyticus*) was applied to assist these two plant species for remediation of Al, an enhancement of 14% was achieved (Purwanti et al., 2020). This shows that the remediation of aluminum can be increased with combination of remediation techniques.

Arsenic

Arsenic (As) is the metalloid with the atomic number 33. It is naturally found in underground water in high concentration in many countries. It is widely distributed in earth's crust. It is not an essential element and toxic to life forms in all its forms. However, the inorganic As^{III} is two to ten times more toxic compared to As^{V}.It can be resulted by volcanic eruptions, or by human activities such as use of pesticides, insecticides, herbicides, dye-stuffs and wood preservatives. Moreover, they are used in pharmaceutical, electronics and industrial manufacturing(Chung et al., 2014). It can reach to human body via drinking the contaminated water, using this water for food preparation, in daily life or for agriculture. It can enter human body via inhalation, skin contact or feeding. It can cause many health problems from skin lesions to cancer of liver, bladder, kidney and skin, cardiovascular diseases and diabetes (WHO, 2018). Arsenic causes changes in DNA synthesis, transcription, translation and cell division, which results in generation of many cancer types in several different tissues (Chung et al., 2014). A lot of research has been going on to determine the effects and behavior of As in microorganisms, plants and human.

The permitted level of As in drinking water was determined to be maximum 10 ug/L by WHO. However, different countries may follow different guidelines which can allow up to 50 ug/L. India is such an example since the earth's crust results in high As concentration in water sources (M. Kumar & Puri, 2012).

Arsenic can accumulate in high levels in agricultural crops when the underground water As level is high. For example, it can be an alarming situation in terms of rice cultivation which accumulates high concentration of As and intense consumption of rice grains cause elevated As transport to human body (Awasthi et al., 2017).

Besides rice, other plants were studied for their accumulation of As. The fern species *Piteris vittata* (ladder brake) was shown to be a hyperaccumulator for arsenic by transporting the As from soil to aboveground parts rather than immobilizing As in roots (Lombi et al., 2002). On the other hand, sunflower (*Helianthus annuus*) was reported to be storing As in roots in high concentration. Therefore, it was suggested to use sunflower for the cleanup of As from soil. Moreover, the oil from sunflower grown on As contaminated soil was suggested to be used safely as biofuel production (Sahito et al., 2021). (Mateo et al., 2019) suggested the phytoremediation method as a feasible application for remediation of arsenic. In order to enhance this method, higher As accumulators should be generated and approaches for this aim has been carried out. For instance, recombinant plants such as *Arabidopsis thaliana* have been generated with genes from plants with high As accumulation capacity (Indriolo et al., 2010). This way cleaning up As from contaminated sites can be enhances with nonagricultural crop plants, or plants with an area of use other than for consumption as food.

Chromium

Chromium (Cr) is a highly toxic metal with an atomic number of 24. In the environment, this heavy metal is mostly found in two valence forms: trivalent chromium and hexavalent chromium. The latter is highly toxic to organisms in every ecosystem, while the former is much less toxic. Chromium is present naturally in rocks. However, as it is heavily used in industries making use of metals, such as automobile industry, chromium can end up in wastes. Besides automobile industry, chromium is used in leather and wood preservation, pigment production. The solid wastes and waste waters may contain chromium which can contaminate air, soil and water systems. Respiratory system is a primary target for hexavalent chromium toxicity. Inhalation of this heavy metal causes health problems from bronchitis to lung cancer (EPA, 2000).

Chromium can as well contaminate food crops grown on contaminated soil. Therefore, the contents of foods and the ways to decrease soil chromium levels should be investigated so that the concentrations in food crops would decrease. The hexavalent chromium is usually reduced to trivalent chromium via metabolic actions, however, the high concentration and long exposure to trivalent chromium is harmful as well (Hamilton et al., 2018). The growth of dock plant (*Rumex patientia* L.) in soil contaminated with 100 mgkg-1 trivalent Cr was investigated. It was revealed that this medicinal aromatic plant was able to accumulate chromium, along with two other heavy metals (Pb and Cd) (Adiloğlu, 2020). In another study, another medicinal aromatic plant *Cirsium vulgare* was used to investigate the hexavalent chromium in this plant. The results revealed that this plant could accumulate hexavalent chromium in its aboveground parts, and the accumulation increased with the chelation of chromium upon addition of EDTA to the soil (Dökmeci & Adiloğlu, 2020).

Along with plants with hyper chromium accumulation capacity, microorganisms can be used for either assistance to plants or they can be applied themselves to remediate chromium form environment. Pushkar et al. (2021) reviewed the chromium tolerant and resistant bacterial strains in their paper. Indeed, recent studies focus on identification of heavy metal resistant bacteria and their metabolism, enzymes and genes which give them the advantage to resist the heavy metals. Some bacteria such as *Bacillus cereus* can resist chromium levels up to 2000 mg/L and can successfully clean all of the chromium found in soil (Banerjee et al., 2019). Moreover, *Micobacterium* sp. Z5 can remove all the hexavalent chromium contamination from the environment. Such microorganisms will contribute to remediation of heavy metals in soil and water sources, and this approach will most probably be more cost effective and easy to apply.

Cadmium

Cadmium is one of the important heavy metals (atomic number 48) frequently found as pollutant in the environment. It is toxic to human health in various forms. It is usually found together with other heavy metals such zinc, copper and lead (Koons & Rajasurya, 2022). Cadmium results from the combustion of fossil fuels, mining activities, and industries such as PVC production, electroplating, pigment production, production of nickel cadmium batteries, and phosphate fertilizers (Rahimzadeh et al., 2017). Volcanic eruptions and forest fires also contribute to cadmium in the atmosphere. It reaches to human body via inhalation, smoking, food ingestion and can give harm to nervous system probably causing Parkinson’s and Alzheimer’s, lungs, bones, kidneys, cardiovascular and reproduction systems (Genchi, Sinicropi, et al., 2020). Moreover, marine organisms such as mollusks and crustaceans can accumulate cadmium and their consumption can result in cadmium exposure of humans via food. Besides, crops such as potatoes and other starchy roots, cereals and vegetables can accumulate this heavy metal which can be the ways to be transferred to human body. World Health Organization suggested that the tolerable limit of cadmium should be 7 μg/kg body weight (WHO, 2003).

The soil contaminated with cadmium should therefore be successfully cleaned in order to decrease the human exposure via food consumption. Cadmium in soil can give serious harm to plants. It can delay seed germination, decrease plant growth and can harm photosynthesis. However, some plants show good tolerance and even resistance to cadmium and can therfore be used for remediation of cadmium. Recent studies address plants such as those belonging to *Brassicaceae*, *Crassulaceae* families (Luo & Zhang, 2021). *Arabidopsis halleri and Noccaea caerulescens* are examples to them (Ó Lochlainn et al., 2011; Stein et al., 2017; Zhao et al., 2006).

Besides plants, microorganisms with cadmium accumulation capabilities were studied. Among the culturable bacteria *Bacillus cereus*, *Bacillus thrungiensis,* and an *Brevundimonas* species were isolated from different areas such as cadmium contaminated paddy soil (Aryal, 2021). *Aspergillus niger*, *Bacillus* and *Streptomyces* species, *Pseudomonas aeruginosa* were also reported to have the capability to accumulate cadmium (Raza et al., 2020). The combination of microorganisms with cadmium remediation capacity with the hyperaccumulator plants might be preferred in the application of remediation techniques to clean up this toxic heavy metal from environments.

Copper

Copper (Cu, atomic number 29) is an essential nutrient element for organisms. Although need in trace amounts, it is vital for the activities of enzymes and hence vital for healthy metabolism. It acts a cofactor for several enzymes such as superoxide dismutase that takes place in redox regulation in cells (Ota & Yokoyama, 2010). Moreover, copper is a structurally required element. An example is mitochondrial enzyme tyrosinase. This enzyme is especially important for bioremediation of phenolic compounds from wastewaters (Agarwal et al., 2019). Copper is an essential micro nutrient element for plants and is required for seed and chlorophyll production.

Although copper is an essential element for life, when exposed in large amounts through skin or drinking water may have acute effects such as gastrointestinal irritation, vomiting, hypotension, and even coma. On the other hand, chromic exposure may result in liver and kidney diseases.

Copper can naturally be found in soils in different concentrations. Industrial processes such as smelting, mining activities and particularly use of pesticides and antifungals for agriculture results in accumulation of copper in soil (Apori et al., 2018). The soil copper can be accumulated in plants, and this causes a problem for agricultural crops which may expose humans with Cu. A remarkable copper accumulator was found to be Helianthus annuus L., which can remove 85% of soil copper (Chen et al., 2018). This important oil crop can accumulate copper mostly in its roots, therefore can be cultivated both for food and phytoremediation. Another edible crop for phytoremediation of copper was *Brassica juncea* L. Indian mustard and amaranth (*Amaranthus paniculatus*) were shown to accumulate soil copper and can be suggested for being quickly growing plants for remediation of this heavy metal (Rahman et al., 2013).

Iron

Iron (atomic number 26) is an essential element for life on earth. It is cofactor of several enzymes, especially the ones taking place in the energy metabolism and the ones for electron transport and it is an essential redox element. Moreover, iron takes places in the structure of many biomolecules required for DNA synthesis and repair (Zhang, 2014). In nature iron is found in Earth’s crust being the fourth most common element. It is usually found as compounds with other elements forming compounds such as hematite. Iron is the basis of steel industry as well as many other industries, especially civil engineering. Such industries and also mining activities can spread iron in the soil and water sources. In a study in Morocco, iron in soil was found to be almost 400 g/kg around an iron mining area. Among the 13 species analyzed, the highest shoot iron concentration was in Ranunculus peltatus Schrank, followed by *Leontodon hispidilus* (Delile) Boiss. *Lamarckia aurea* L. (Moench) and *Carthamus lanatus* L. were also found to be significant iron accumulators (Nouri & Haddioui, 2016).

Since it is an essential element, it is present in many fertilizers to enrich the soil. However, unconscious fertilizer application increases iron level to toxic levels in soil. The increased iron level can accumulate in plants grown on such soils. This situation was shown to decrease health benefits of *Ocimum basilicum* as the iron concentration in soil increases iron concentration in plant (Gürgan & Adiloğlu, 2021). There are several other studies revealing the increased iron levels in plants upon high soil iron concentrations (Malayeri et al., 2008). *Centella asiatica* plant was used to test the remediation of soil contaminated with iron. Its root was the optimum place to accumulate iron followed by leaves and stems with different concentrations of iron accumulation (Ul et al., 2016)

The above studies and many others revealed that plants can absorb iron from soil in a toxic level. The fertilizer production can lead accumulation of high amounts of iron contamination in agricultural crops. When the ingestion of 60 mg/kg and higher iron occurs in people, it can cause severe toxicity that can results in death. Less severe toxicity can cause nausea, vomiting, diarrhea, perforation of gastric mucosa, and also mitochondrial disruption causing free radial formation and cell death (Yuen & Gossman, 2022). Moreover, high iron exposure can cause suicide attempts. Therefore, although iron fertilization seems innocent, it can result severe human health problems, and hence the iron contamination in soil should be remediated.

Lead

Lead (Pb) is one of the major heavy metals with a a high toxicity. The element with atomic number 82, has no level to be non-harmful to human health. It is especially very problematic for young children. Priorly, it harms nervous system and brain and causes death. But even in low exposure it causes renal disorders, reduced IQ levels in children, reduced attention, anemia, hypertension, inhibition of vitamin D synthesis, and maintenance of cell membrane and DNA transcription (Wani et al., 2015; WHO, 2022). The Center for Disease Control and Prevention suggested to take urgent measures for children with a blood lead level of equal to or greater than 5 μg/dL (CDC, 2012).

Human exposure can occur as a result of industrial activities such as lead smelting, pigment industry, recycling of batteries, usage of leaded gas, and even from book printing (Wani et al., 2015). Lead in air resulting from fuel consumption can contaminate soil and it is very persistent (EPA, 2002). It can be transferred to crops and can reach to human body through ingestion (Kumar et al 2020). Therefore, lead remediation from sol is critical. It was found that remediation of soil lead decreases the blood lead levels in children which is very vital for physical and mental health of children (Dobrescu et al., 2022). Plants were shown to be used to remediate soil and water sources from heavy metals, lead included.

Atriplex halimus a perennial shrub with a long stem, well known for its tolerance and resistance to drought and salinity: good phytostabilization which is accumulation of Pb in roots and limited phytoextraction (Acuña et al., 2021). Another shrub, *Sesbania drummondii* was shown to be hyper accumulator for lead in its shoots (Sahi et al., 2002). *Beta vulgaris* (sugar beet) grown on lead contaminated soil in nature was shown to contain a high concentration of lead, which may suggest to use this plant to clean up lead from to soil (Murthy et al., 2012). Experimental studies to determine the lead accumulation of different plant species revealed that *Pistia stratiotes* can remove 90% of lead in the solution. Similarly, the water hyacinth *Eichhornia crassipes. Eichhornia crassipes* was determined to have the capability to remove more than 98% of the lead in water bodies (Q. Li et al., 2016). Many other examples can be given for utilizing plants for bioremediaiton of lead contamination (A. Kumar et al., 2020)

Mercury

Mercury is a liquid metal with the atomic number of 80. It is naturally present in Earth’s crust and can be spread to the environment from volcanic eruptions, rock weathering, col powered power plants, burning of coal, mining of mercury and gold. It is used in thermometers, batteries, barometers, light bulbs, dental filling and some cosmetics. Using such products might expose people to mercury (R. Li et al., 2017; WHO, 2017b). Exposure to mercury causes serious health problems. Even a low dose is toxic, especially to central and peripheral nervous system. Consumption of fish with accumulated mercury is a way for mercury entrance to human body.

Besides these, mercury can enter human body via foods such as vegetables and grains. Especially crops produce on lands near coal power plants are reported to be contaminated with mercury. Almost 80% of crops near the coal-fired power plants were shown to contain mercury levels exceeding the tolerable limits (R. Li et al., 2017). The plants reported to contain mercury were spinach, cowpea, rice, lettuce and amaranth. In these plants, leaves contained higher mercury levels than roots, hopefully the levels decreased after rinsing of the vegetables. Such researches are important as warning for agricultural activities around coal fired power plants.

These researches also reveal that mercury can be accumulated in plant bodies, which suggests the use of plants for cleaning up the mercury contamination from soil. Since mercury possesses serious health problems in even low doses, several studies have been conducted for the remediation of mercury from soil and to determine Hg hyper accumulator plants. Due to time and cost effectiveness, perennial plants are suggested for phytoremediation, and indeed *Eremochloa ciliaris* grass and indeed it was found or *Erato polymnioides* have been shown to accumulate mercury well (Chamba et al., 2017; Cho-Ruk et al., 2006; Liu et al., 2020). In another study, another grass from Poaceae family (*Lolium perenne*) was shown to accumulate mercury in its stem and its performance increased by the mycorrhizal symbiosis but this time the heavy metal accumulation happened not in stems but in roots (Leudo et al., 2020). Apart from food crops, an energy crop was tested on mercury and cadmium accumulation capacity. *Miscanthus x giganteus* was shown to be a good candidate for remediation of mercury and cadmium heavy metals from the soil which will later be evaluated for biomass energy production (Zgorelec et al., 2020).

Nickel

Nickel (Ni, atomic number 28) is widely distributed in earth's crust. It is an essential element for plants, microorganisms and some animals since it is a cofactor of some enzymes. However, in high concentrations it exerts toxic effects to plants and results in decrease in enzyme activities, reduced chlorophyll synthesis, etc (Genchi, Carocci, et al., 2020). As a result of high dose or long exposure it exerts toxicity in humans, too. Nickel is well known for its allergic effects when used in inexpensive jewelry. Apart from this, it can cause more serious health problems such as cancer, lung fibrosis, gastrointestinal dysfunctions and cardiovascular diseases (Genchi, Carocci, et al., 2020). Human exposure can be due to use of jewelry, cell phones, keys, paper clips, metal parts of clothings such as zippers, orthodontic braces etc. Nickel can also be released to the environment from several industries such as pigment production, power plants, food processing. Food is actually a major source for nickel exposure. Moreover, smoking tobacco and drinking water can be sources for nickel exposure (Agency for toxic substances and disease registry, 2005).

Soil is contaminated with nickel in a lot of places all over the world. Sajad et al. (2020) studied 61 plant species in nature in Pakistan and their nickel accumulations in their stems, roots, and in the soil. This comprehensive study revealed that *Filago hurdwarica* and *Xanthium strumarium* L. from *Asteraceae* family and *Medicago lupulina* L. were very successful for accumulating nickel in their roots and therefore for phytostabilization of nickel. On the other hand, *Asplenium dalhousiae, Isatis tinctoria* L., *Bryophyllum daigremontianum, Rosularia adenotricha*, and *Iris germanica* L. remarkably accumulated nickel of higher than 100 mg/kg dry weight in their shoots. They suggested that 26 of the 61 plants were feasible for nickel remediation. In another study, a naturally growing shrub species in Qatar, *Tetraena qataranse* was shown to accumulate nickel in its roots and good at phytostabilization of this heavy metal, along with copper and chromium (Usman et al., 2019). Such studies reveal that soil contamination with nickel can be remediated with several plant species.

Zinc

Zinc is both an essential element for plants and animals, and also a heavy metal, whose benefit or harm is dose dependent. The atomic number of zinc is 30. It is vital for plant growth and human health, and exerts toxic effects when found in large concentrations.

The form of zinc is dependent on the soil properties. For example, Zn_{2+} is the most dominant form in pH range of 0-8, while in the pH range of 9-14 the most dominant form is $ZnOH^{+}$. Soil pH is the determinant for the deficiency, sufficiency or toxicity of Zn for plants (Natasha et al., 2022). Therefore, in acidic soils Zn toxicity is more frequent for plants than its deficiency. For edible plants, Zn content is recommended to be 60 mg/g by(FAO/WHO, 2001). Zinc toxicity in plants shows itself as decreased growth, disturbed enzymatic activities and pigment formation.

Zinc contaminates soil via rock weathering, volcanic eruptions, forest fires, mining, waste-waters, fertilizers, etc. Humans can be exposed to zinc via long term usage of contaminated food and beverages and it inhibits Fe and Co uptake and hence leads to decreased metabolic efficiency in humans (Agency for Toxic Substances and Disease Registry, 2005). Moreover, high zinc concentrations decrease agricultural crop production due to decreased metabolic efficiencies in plants, too (Kaur & Garg, 2021).

It is, therefore, important to inhibit zinc contamination and build up in soil. In already contaminated areas, phytoremediation can be a preferred technique. Several plant species have been tested for their zinc accumulation. Some examples are the penny grass *Thlaspi caerulescens* (Xie et al., 2009), mown turfgrass *Festuca rubra* (Gómez et al., 2020), 2020), (Dmuchowski et al., 2014), *Arabidopsis halleri* (Isaure et al., 2015), and *Brachiaria* *ramose* (Lakshmi et al., 2013), *Betula pendula* Roth (Dmuchowski et al., 2014), *Salix pedicellata* (Amdoun et al., 2020), *C. didymus* (Sidhu et al., 2016). The effort to understand the metabolism of hyper-accumulation of zinc, as well as other heavy metals, will lead scientist to generate better accumulators. Countless researches have been conducted on both to determine accumulation capacities of plants and to identify the mechanisms behind the hyper accumulation of heavy metals.

Conclusion

Heavy metals are very important for the health of environments, plants, animals and humans. The natural and anthropogenic activities can result in accumulation of high concentrations of heavy metals in soil and water sources. They can enter the vegetative and edible parts of plants, and via, directly or indirectly, ingestion by animals and humans, they can enter the food chain and reach to human body. Depending on the heavy metal species and its concentration, it may result even in death. Therefore, the soil heavy metal contaminations should be determined well locally and globally and remediation of soil and water sources from heavy metals should be carried out in a well-planned manner. Microorganisms and plant species can be employed for the remediation of them. Several different bacterial, fungal, algal species and many agricultural and nonagricultural plant species have been tested and determined for their performance for heavy metal accumulation. Many genetic studies have been conducted to understand the mechanisms behind heavy metal tolerance and resistance. Such studies will lead scientist to generate better hyperaccumulators and better microorganismal assistance for heavy metal remediation.

REFERENCES

Acuña, E., Castillo, B., Queupuan, M., Casanova, M., & Tapia, Y. (2021). Assisted phytoremediation of lead contaminated soil using Atriplex halimus and its effect on some soil physical properties. *International Journal of Environmental Science and Technology*, *18*(7), 1925–1938. https://doi.org/10.1007/S13762-020-02978-5/TABLES/8

Adiloğlu, S. (2020). Interaction of Some Heavy Metals with Copper Content in Dock Plant. *2020 KSU J. Agric Nat*, *23*(4), 1078–10845. https://doi.org/10.18016/ksutarimdoga.vi.689443

Agarwal, P., Singh, M., Singh, J., & Singh, R. P. (2019). Microbial Tyrosinases: A Novel Enzyme, Structural Features, and Applications. *Applied Microbiology and Bioengineering*, 3–19. https://doi.org/10.1016/B978-0-12-815407-6.00001-0

Agency for toxic substances and disease registry. (2005). *Public Health Statement for Nickel*.

Agency for Toxic Substances and Disease Registry. (2005). *Toxicological Profile for Nickel*.

Agency for Toxic Substances, & and Disease Registry. (2011). *ToxGuide TM for Toxicokinetics and Normal Human Levels Biomarkers/Environmental Levels Sources of Exposure*. http://www.atsdr.cdc.gov/toxprofiles/index.asp

Alori, E., & Fawole, O. (2012). Phytoremediation of Soils Contaminated with Aluminium and Manganese by Two Arbuscular Mycorrhizal Fungi. *Journal of Agricultural Science*, *4*(8), p246. https://doi.org/10.5539/JAS.V4N8P246

Amdoun, R., Bendifallah, N., Sahli, F., Moustafa, K., Hefferon, K., Makhzoum, A., & Khelifi, L. (2020). Improving zinc phytoremediation characteristics in Salix pedicellata with a new acclimation approach. *International Journal of Phytoremediation*, *22*(7), 745–754. https://doi.org/10.1080/15226514.2019.1708862

Apori, O. S., Hanyabui, E., Asiamah, Y. J., & Rangel, J. H. (2018). Remediation Technology for copper contaminated soil : A review Remediation Technology for Copper Contaminated Soil: A Review.

Asian Soil Research Journal, *1*(3), 1–7. https://doi.org/10.9734/ASRJ/2018/45322

Aryal, M. (2021). A comprehensive study on the bacterial biosorption of heavy metals: Materials, performances, mechanisms, and mathematical modellings. *Reviews in Chemical Engineering*, *37*(6), 715–754. https://doi.org/10.1515/REVCE-2019-0016/XML

Ashenef, A. (2014). Essential and toxic metals in tea (Camellia sinensis) imported and produced in Ethiopia. *Food Additives & Contaminants. Part B, Surveillance*, *7*(1), 30–36. https://doi.org/10.1080/19393210.2013.831951

Awasthi, S., Chauhan, R., Srivastava, S., & Tripathi, R. D. (2017). The journey of arsenic from soil to Grain in Rice. *Frontiers in Plant Science*, *8*, 1007. https://doi.org/10.3389/FPLS.2017.01007/BIBTEX

Banerjee, S., Misra, A., Chaudhury, S., & Dam, B. (2019). A Bacillus strain TCL isolated from Jharia coalmine with remarkable stress responses, chromium reduction capability and bioremediation potential. *Journal of Hazardous Materials*, *367*, 215–223. https://doi.org/10.1016/J.JHAZMAT.2018.12.038

Bojórquez-Quintal, E., Escalante-Magaña, C., Echevarría-Machado, I., & Martínez-Estévez, M. (2017). Aluminum, a friend or foe of higher plants in acid soils. *Frontiers in Plant Science*, *8*, 1767. https://doi.org/10.3389/FPLS.2017.01767/BIBTEX

CDC. (2012). *Low Level Lead Exposure Harms Children: A Renewed Call for Primary Prevention Report* .

Chamba, I., Rosado, D., Kalinhoff, C., Thangaswamy, S., Sánchez-Rodríguez, A., & Gazquez, M. J. (2017). Erato polymnioides - A novel Hg hyperaccumulator plant in ecuadorian rainforest acid soils with potential of microbe-associated phytoremediation. *Chemosphere*, *188*, 633–641. https://doi.org/10.1016/J.CHEMOSPHERE.2017.08.160

Chen, S., Zhang, H., Wang, X., -, al, Hammadi, H. J., Faiath, S. E., Azzam -, M. R., Mahardika, G., Rinanti, A., & Fachrul, M. F. (2018). Phytoremediation of heavy metal copper (Cu2+) by sunflower (Helianthus annuus l.). *IOP Conference Series: Earth and Environmental Science*, *106*(1), 012120. https://doi.org/10.1088/1755-1315/106/1/012120

Cho-Ruk, K., Kurukote, J., Supprung, P., & Vetayasuporn, S. (2006). Perennial plants in the phytoremediation of lead-contaminated soils. *Biotechnology*, *5*(1), 1–4. https://doi.org/10.3923/BIOTECH.2006.1.4

Chung, J. Y., Yu, S. do, & Hong, Y. S. (2014). Environmental Source of Arsenic Exposure. *Journal of Preventive Medicine and Public Health*, *47*(5), 253. https://doi.org/10.3961/JPMPH.14.036

Dmuchowski, W., Gozdowski, D., Bragoszewska, P., Baczewska, A. H., & Suwara, I. (2014). Phytoremediation of zinc contaminated soils using silver birch (Betula pendula Roth). *Ecological Engineering*, *71*, 32–35. https://doi.org/10.1016/J.ECOLENG.2014.07.053

Dobrescu, A. I., Ebenberger, A., Harlfinger, J., Griebler, U., Klerings, I., Nußbaumer-Streit, B., Chapman, A., Affengruber, L., & Gartlehner, G. (2022). Effectiveness of interventions for the remediation of lead-contaminated soil to prevent or reduce lead exposure - A systematic review. *Science of The Total Environment*, *806*, 150480. https://doi.org/10.1016/J.SCITOTENV.2021.150480

Dökmeci, A. H., & Adiloğlu, S. (2020). The Phytoremediation of Chromium from Soil Using Cirsium Vulgare and the Health Effects. *Biosciences Biotechnology Research Asia*, *17*(03), 535–541. https://doi.org/10.13005/bbra/2857

EPA. (2000). *Chromium Compounds*.

EPA. (2002). *Basic information about Lead Air Pollution*.

Exley, C. (2017). Aluminum Should Now Be Considered a Primary Etiological Factor in Alzheimer's Disease. *Journal of Alzheimer's Disease Reports*, *1*(1), 23–25. https://doi.org/10.3233/ADR-170010

Exley, C., & Clarkson, E. (2020). Aluminium in human brain tissue from donors without neurodegenerative disease: A comparison with Alzheimer's disease, multiple sclerosis and autism. *Scientific Reports 2020 10:1*, *10*(1), 1–7. https://doi.org/10.1038/s41598-020-64734-6

FAO/WHO. (2001). *Codex Alimentarius Commission Food Additives and Contaminants*.

Genchi, G., Carocci, A., Lauria, G., Sinicropi, M. S., & Catalano, A. (2020). Nickel: Human Health and Environmental Toxicology. *International Journal of Environmental Research and Public Health*, *17*(3). https://doi.org/10.3390/IJERPH17030679

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The Effects of Cadmium Toxicity. *International Journal of Environmental Research and Public Health*, *17*(11). https://doi.org/10.3390/IJERPH17113782

Gómez, J., García, S., Esteban, E., Zornoza, P., & Carpena, R. O. (2020). The role of root organic acids in the tolerance of Festuca rubra to zinc, lead and cadmium. *Https://Doi.Org/10.1080/02772248.2020.1791866*, 399–414. https://doi.org/10.1080/02772248.2020.1791866

Gürgan, M., & Adiloğlu, S. (2021). Increasing concentrations of iron fertilizer affect antibacterial activity of basil (Ocimum basilicum L.). *Industrial Crops and Products*, *170*, 113768. https://doi.org/10.1016/J.INDCROP.2021.113768

Hamilton, E. M., Young, S. D., Bailey, E. H., & Watts, M. J. (2018). Chromium speciation in foodstuffs: A review. *Food Chemistry*, *250*, 105–112. https://doi.org/10.1016/J.FOODCHEM.2018.01.016

Indriolo, E., Na, G. N., Ellis, D., Salt, D. E., & Banks, J. A. (2010). A vacuolar arsenite transporter necessary for arsenic tolerance in the arsenic hyperaccumulating fern Pteris vittata is missing in flowering plants. *The Plant Cell*, *22*(6), 2045–2057. https://doi.org/10.1105/TPC.109.069773

Isaure, M. P., Huguet, S., Meyer, C. L., Castillo-Michel, H., Testemale, D., Vantelon, D., Saumitou-Laprade, P., Verbruggen, N., & Sarret, G. (2015). Evidence of various mechanisms of Cd sequestration in the hyperaccumulator Arabidopsis halleri, the non-accumulator Arabidopsis lyrata, and their progenies by combined synchrotron-based techniques. *Journal of Experimental Botany*, *66*(11), 3201–3214. https://doi.org/10.1093/JXB/ERV131

Kanwar, V. S., Sharma, A., Srivastav, A. L., & Rani, L. (2020). Phytoremediation of toxic metals present in soil and water environment: a critical review. *Environmental Science and Pollution Research 2020 27:36*, *27*(36), 44835–44860. https://doi.org/10.1007/S11356-020-10713-3

Kaur, H., & Garg, N. (2021). Zinc toxicity in plants: a review. *Planta*, *253*(6). https://doi.org/10.1007/S00425-021-03642-Z

Koons, A. L., & Rajasurya, V. (2022). *Cadmium Toxicity*. StatPearls Publishing.

Kumar, A., Kumar, A., Cabral-Pinto, M., Chaturvedi, A. K., Shabnam, A. A., Subrahmanyam, G., Mondal, R., Gupta, D. K., Malyan, S. K., Kumar, S. S., Khan, S. A., & Yadav, K. K. (2020). Lead Toxicity: Health Hazards, Influence on Food Chain, and Sustainable Remediation Approaches. *International Journal of Environmental Research and Public Health 2020, Vol. 17, Page 2179*, *17*(7), 2179. https://doi.org/10.3390/IJERPH17072179

Kumar, M., & Puri, A. (2012). A review of permissible limits of drinking water. *Indian Journal of Occupational and Environmental Medicine*, *16*(1), 40. https://doi.org/10.4103/0019-5278.99696

Lakshmi, P. M., Jaison, S., Muthukumar, T., & Muthukumar, M. (2013). Assessment of metal accumulation capacity of Brachiaria ramosa collected from cement waste dumping area for the remediation of metal contaminated soil. *Ecological Engineering*, *60*, 96–98. https://doi.org/10.1016/J.ECOLENG.2013.07.043

Leudo, A. M., Cruz, Y., Montoya-Ruiz, C., Delgado, M. del P., & Saldarriaga, J. F. (2020). Mercury Phytoremediation with Lolium perenne-Mycorrhizae in Contaminated Soils. *Sustainability 2020, Vol. 12, Page 3795*, *12*(9), 3795. https://doi.org/10.3390/SU12093795

Li, Q., Chen, B., Lin, P., Zhou, J., Zhan, J., Shen, Q., & Pan, X. (2016). Adsorption of heavy metal from aqueous solution by dehydrated root powder of long-root Eichhornia crassipes. *International Journal of Phytoremediation*, *18*(2), 103–109. https://doi.org/10.1080/15226514.2014.898017

Li, R., Wu, H., DIng, J., Fu, W., Gan, L., & Li, Y. (2017). Mercury pollution in vegetables, grains and soils from areas surrounding coal-fired power plants. *Scientific Reports 2017 7:1*, *7*(1), 1–9. https://doi.org/10.1038/srep46545

Liu, Z., Chen, B., Wang, L. ao, Urbanovich, O., Nagorskaya, L., Li, X., & Tang, L. (2020). A review on phytoremediation of mercury contaminated soils. *Journal of Hazardous Materials*, *400*, 123138. https://doi.org/10.1016/J.JHAZMAT.2020.123138

Lombi, E., Zhao, F. J., Fuhrmann, M., Ma, L. Q., & McGrath, S. P. (2002). Arsenic distribution and speciation in the fronds of the hyperaccumulator

Pteris vittata. *New Phytologist*, *156*(2), 195–203. https://doi.org/10.1046/J.1469-8137.2002.00512.X

Luo, J. S., & Zhang, Z. (2021). Mechanisms of cadmium phytoremediation and detoxification in plants. *The Crop Journal*, *9*(3), 521–529. https://doi.org/10.1016/J.CJ.2021.02.001

Malayeri, B. E., Chehregani, A., Yousefi, N., & Lorestani, B. (2008). Identification of the hyper accumulator plants in copper and iron mine in Iran. *Pakistan Journal of Biological Sciences : PJBS*, *11*(3), 490–492. https://doi.org/10.3923/PJBS.2008.490.492

Mateo, C., Navarro, M., Navarro, C., Leyva, A., Mateo, C., Navarro, M., Navarro, C., & Leyva, A. (2019). Arsenic Phytoremediation: Finally a Feasible Approach in the Near Future. *Environmental Chemistry and Recent Pollution Control Approaches*. https://doi.org/10.5772/INTECHOPEN.88207

Mehra, A., & Baker, C. L. (2007). Leaching and bioavailability of aluminium, copper and manganese from tea (Camellia sinensis). *Food Chemistry*, *100*(4), 1456–1463. https://doi.org/10.1016/J.FOODCHEM.2005.11.038

Murthy, V. N. Y., Ramesh, H. L., Yogananda, V. N., & *, M. (2012). *Assessment of Heavy Metal Contamination in Green Leafy Vegetables Grown in Bangalore Urban District of Karnataka Screening of Medicinal Plants for Secondary Metabolites View project Assessment of Heavy Metal Contamination in Green Leafy Vegetables Grown in Bangalore Urban District of Karnataka* (Vol. 6). Online. https://www.researchgate.net/publication/309760358

Natasha, N., Shahid, M., Bibi, I., Iqbal, J., Khalid, S., Murtaza, B., Bakhat, H. F., Farooq, A. B. U., Amjad, M., Hammad, H. M., Niazi, N. K., & Arshad, M. (2022). Zinc in soil-plant-human system: A data-analysis review. *Science of The Total Environment*, *808*, 152024. https://doi.org/10.1016/J.SCITOTENV.2021.152024

Nouri, M., & Haddioui, A. (2016). Human and animal health risk assessment of metal contamination in soil and plants from Ait Ammar abandoned iron mine, Morocco. *Environmental Monitoring and Assessment*, *188*(1), 1–12. https://doi.org/10.1007/S10661-015-5012-6/TABLES/7

Ó Lochlainn, S., Bowen, H. C., Fray, R. G., Hammond, J. P., King, G. J., White, P. J., Graham, N. S., & Broadley, M. R. (2011). Tandem Quadruplication of HMA4 in the Zinc (Zn) and Cadmium (Cd) Hyperaccumulator Noccaea caerulescens. *PLOS ONE*, *6*(3), e17814. https://doi.org/10.1371/JOURNAL.PONE.0017814

Ota, M., & Yokoyama, M. (2010). Comprehensive Natural Products II. *Comprehensive Natural Products II*, 317–349. http://www.sciencedirect.com:5070/referencework/9780080453828/comprehensive-natural-products-ii

Panda, S. K., Baluska, F., & Matsumoto, H. (2009). Aluminum stress signaling in plants. *Plant Signaling & Behavior*, *4*(7), 592. https://doi.org/10.4161/PSB.4.7.8903

Purwanti, I. F., Obenu, A., Tangahu, B. V., Kurniawan, S. B., Imron, M. F., & Abdullah, S. R. S. (2020). Bioaugmentation of Vibrio alginolyticus in phytoremediation of aluminium-contaminated soil using Scirpus grossus and Thypa angustifolia. *Heliyon*, *6*(9), e05004. https://doi.org/10.1016/J.HELIYON.2020.E05004

Pushkar, B., Sevak, P., Parab, S., & Nilkanth, N. (2021). Chromium pollution and its bioremediation mechanisms in bacteria: A review. *Journal of Environmental Management*, *287*, 112279. https://doi.org/10.1016/J.JENVMAN.2021.112279

Rahimzadeh, M. R., Rahimzadeh, M. R., Kazemi, S., & Moghadamnia, A. A. (2017). Cadmium toxicity and treatment: An update. *Caspian Journal of Internal Medicine*, *8*(3), 135. https://doi.org/10.22088/CJIM.8.3.135

Rahman, M. M., Azirun, S. M., & Boyce, A. N. (2013). Enhanced Accumulation of Copper and Lead in Amaranth (Amaranthus paniculatus), Indian Mustard (Brassica juncea) and Sunflower (Helianthus annuus). *PLOS ONE*, *8*(5), e62941. https://doi.org/10.1371/JOURNAL.PONE.0062941

Raj, J., & Rebecca, L. J. (2014). Phytoremediation of aluminium and lead using Raphanus sativus, Vigna radiata and Cicer arietinum. *Available Online Www.Jocpr.Com Journal of Chemical and Pharmaceutical Research*, *6*(5), 1148–1152. www.jocpr.com

Raza, A., Habib, M., Kakavand, S. N., Zahid, Z., Zahra, N., Sharif, R., & Hasanuzzaman, M. (2020). Phytoremediation of Cadmium:

Physiological, Biochemical, and Molecular Mechanisms. *Biology, 9*(7), 1–46. https://doi.org/10.3390/BIOLOGY9070177

Sahi, S. v., Bryant, N. L., Sharma, N. C., & Singh, S. R. (2002). Characterization of a Lead Hyperaccumulator Shrub, Sesbania drummondii. *Environmental Science and Technology, 36*(21), 4676–4680. https://doi.org/10.1021/ES020675X

Sahito, Z. A., Zehra, A., Tang, L., Ali, Z., Laeeq ur Rehman Hashmi, M., Bano, N., Ullah, M. A., He, Z., & Yang, X. (2021). Arsenic and mercury uptake and accumulation in oilseed sunflower accessions selected to mitigate co-contaminated soil coupled with oil and bioenergy production. *Journal of Cleaner Production, 291*, 125226. https://doi.org/10.1016/J.JCLEPRO.2020.125226

Sajad, M. A., Khan, M. S., Bahadur, S., Shuaib, M., Naeem, A., Zaman, W., & Ali, H. (2020). Nickel phytoremediation potential of some plant species of the Lower Dir, Khyber Pakhtunkhwa, Pakistan. *Limnological Review, 20*(1), 13–22. https://doi.org/10.2478/LIMRE-2020-0002

Sidhu, G. P. S., Singh, H. P., Batish, D. R., & Kohli, R. K. (2016). Effect of lead on oxidative status, antioxidative response and metal accumulation in Coronopus didymus. *Plant Physiology and Biochemistry, 105*, 290–296. https://doi.org/10.1016/J.PLAPHY.2016.05.019

Stein, R. J., Höreth, S., de Melo, J. R. F., Syllwasschy, L., Lee, G., Garbin, M. L., Clemens, S., & Krämer, U. (2017). Relationships between soil and leaf mineral composition are element-specific, environment-dependent and geographically structured in the emerging model Arabidopsis halleri. *New Phytologist, 213*(3), 1274–1286. https://doi.org/10.1111/NPH.14219

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy Metals Toxicity and the Environment. *EXS, 101*, 133. https://doi.org/10.1007/978-3-7643-8340-4_6

Ul, I., Bhat, H., Mauris, N., Khanam, Z., Kelantan, M., & Kelantan, M. ; (2016). Phytoremediation of iron from red soil of tropical region by using Centella asiatica. *Http://Dx.Doi.Org/10.1080/15226514.2016.1156637, 18*(9), 918–923. https://doi.org/10.1080/15226514.2016.1156637

Usman, K., Al-Ghouti, M. A., & Abu-Dieyeh, M. H. (2019). The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant Tetraena qataranse. *Scientific Reports 2019 9:1*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-42029-9

Wang, C., & Cui, Y. (2019). Recognition of a New Cr(VI)-Reducing Strain and Study of the Potential Capacity for Reduction of Cr(VI) of the Strain. *BioMed Research International*, 1–9. https://doi.org/10.1155/2019/5135017

Wang, S., & Shi, X. (2001). Molecular mechanisms of metal toxicity and carcinogenesis. *Molecular and Cellular Biochemistry 2001 222:1*, *222*(1), 3–9. https://doi.org/10.1023/A:1017918013293

Wani, A. L., Ara, A., & Usmani, J. A. (2015). Lead toxicity: a review. *Interdisciplinary Toxicology*, *8*(2), 55. https://doi.org/10.1515/INTOX-2015-0009

WHO. (2003). *Cadmium Review*.

WHO. (2017a). *Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum*. http://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/

WHO. (2017b). *Mercury and health*.

WHO. (2018). *Arsenic*.

WHO. (2022). *Lead Poisoning*.

Xie, H. L., Jiang, R. F., Zhang, F. S., McGrath, S. P., & Zhao, F. J. (2009). Effect of nitrogen form on the rhizosphere dynamics and uptake of cadmium and zinc by the hyperaccumulator Thlaspi caerulescens. *Plant and Soil*, *318*(1–2), 205–215. https://doi.org/10.1007/S11104-008-9830-Y/FIGURES/4

Yuen, H.-W., & Gossman, W. G. (2022). Iron Toxicity. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK459224/

Zgorelec, Z., Bilandzija, N., Knez, K., Galic, M., & Zuzul, S. (2020). Cadmium and Mercury phytostabilization from soil using Miscanthus × giganteus. *Scientific Reports 2020 10:1*, *10*(1), 1–10. https://doi.org/10.1038/s41598-020-63488-5

Zhang, C. (2014). Essential functions of iron-requiring proteins in DNA replication, repair and cell cycle control. *Protein & Cell*, *5*(10), 750. https://doi.org/10.1007/S13238-014-0083-7

Zhao, F. J., Jiang, R. F., Dunham, S. J., & McGrath, S. P. (2006). Cadmium uptake, translocation and tolerance in the hyperaccumulator Arabidopsis halleri. *The New Phytologist*, *172*(4), 646–654. https://doi.org/10.1111/J.1469-8137.2006.01867.X

CHAPTER 14

DETERMINATION OF THE CONTACT AND FUMIGANT ACTIVITY OF *Zingiber officinale* ROSCOE (ZINGIBERALES: ZINGIBERACEAE) ESSENTIAL OIL AGAINST *Acanthoscelides obtectus* (SAY) (COLEOPTERA: CHRYSOMELIDAE: BRUCHINAE) UNDER LABORATORY CONDITIONS

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INTRODUCTION

Stored products are threatened by physical factors such as temperature, humidity, ventilation, air quality, air components, as well as rodents, insects, mites and fungi (Dizlek, 2012). It is reported that post-harvest products are damaged by 14% in storage (Burçak et al., 2015). *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae) is one of the main pests causing crop losses in the storage of legumes and causes significant damages all over the world. *A.obtectus* is common in almost every region of our country and causes economic losses by feeding on bean seeds. As a result of the damage caused by eating the grains, they cause 80-90% damage in the germination rates of the grains (Anonymous, 2008). Cultural, mechanical and chemical control methods are used to reduce insect damage to stored products. However, due to the inadequacy of cultural and mechanical methods, the use of insecticides is widespread as faster and more effective results are needed. Organic phosphorus, pyrethroid group insecticides, and fumigants are among the most used chemicals in the control against storage pests. Due to reasons such as residue, environmental pollution, resistance and high toxicity caused by these insecticides used, it has been revealed that alternative control methods should be developed in the control against stored grain pests (Alkan and Gökçe, 2012; Karakoç et al., 2013; Ertürk et al., 2017). In recent years, significant developments have been made in using biopesticides in pest management. Essential oils contained in plants are effective in defence strategies against many plant pests. Essential oils have the advantages of having a broad spectrum of effects, low toxicity to non-target organisms, low residue risk and potential for commercialisation. By 2030, the pesticides currently in active use will be restricted and therefore alternatives that can be effective in pest control need to be placed on the market. Many studies are carried out against storage pests in search of environmentally friendly plant-based insecticides among alternative control methods and to develop new effective products that can be used against pest species (Karakoç et al., 2006; Alkan and Gökçe, 2012; Karakoç et al., 2013; Çetin et al., 2015).

Plants show pesticide properties against pests by their secondary metabolites such as terpenoids and alkaloids. Extracts or essential oils obtained from roots, tubers, stems, leaves or flowers of plants belonging to families such as Lamiaceae, Meliaceae, Labiatae, Annonaceae, Malvaceae, Myristicaceae and Zingiberaceae are currently used as biopesticides against many plant protection agents in fields, gardens and storage (Dimetry, 2014). Zingiberaceae family is one of the families in which plants with biopesticide properties are found. Ginger from the Zingiberaceae family has a perennial tuber and rhizome roots. It has been used for centuries as a spice and medicinal plant both in dry and wet parts for snake bites, as a respiratory regulator, rheumatic effects, preventing clotting, preventing cholesterol and toothache, and as a spice for the flavour it adds to meals (Kaplan, 2005; Bayar Uysal, 2020). The first record of the biopesticide properties of ginger is that African natives ate ginger to repel mosquitoes (Kaplan, 2005).

Suthisut et al. (2011) evaluated essential oils and main components of rhizomes of three plants belonging to Zingiberaceae family on storage pests *Sitophilus zeamais* and *Tribolium castaneum*, and on parasitoids *Anisopteromalus calandrae* and *Trichogramma deion*. It was determined that parasitoids were susceptible to essential oils and *T.castaneum* was more susceptible to essential oils than *S.zeamais*. Loni and Panahi (2015) in their study tested the essential oil of *Z.officinale* and *M.pulegium* against *Callosobruchus maculatus* and it was found that *Z.officinale* was more effective than *M.pulegium.* They suggested that it has the potential to be used as a fumigant in storage. Ahmad et al. (2016) examined *P. nigrum, N. tabacum, C. longa* and *Z. officinale* plant extracts against *Rhyzopertha dominica*. The results showed that *P. nigrum* had the highest insecticidal effect and *Z. officinale* had the least effect. Mohammadi Nori et al. (2018) have determined the ovicidal and larvicidal activity of nine plant powders against *C.maculatus*. They determined that plant powders of *Z.officinale* and *M.pulegium* showed the highest larvicidal and ovicidal activity.

Regnault-Roger et al. (1993) used essential oils obtained from 22 plants belonging to Labiaceae, Myrtaceae, Lauraceae, Graminaceae, Umbelliferae, Lauraceae, Myristicaceae, and Rutacae families against *Acanthoscelides obtectus*. They have determined that most of the plants belonging to the Labiaceae family have fumigant activity. The most effective plants were found to be *Origanum marjorana* and *Thymus serpyllum*. Considering the studies, it was determined that *Z.officinale* essential oil was not examined against *A.obtectus*.

In this study, contact and fumigant activity of *Zingiber officinale* essential oil against *Acanthoscelides obtectus* was determined.

MATERIAL AND METHOD

Rearing of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae)

Acanthoscelides obtectus used in the experiment were obtained from the stock culture of Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Plant Protection. *A. obtectus* adults were reared in 1 L glass jars and the jars were covered with muslin with the use of rubber bands. To obtain adult populations of the same age, 1 L glass jars were filled 1/2 with clean dry beans (*Phaseolus vulgaris* L.). The insects were kept for 7 days for putting eggs and then insects were removed from the beans. The beans with eggs in glass jars were kept in an incubator at 27±2°C and 60%. For the experiment 1-week-old adults were used.

Extracting *Zingiber officinale* Roscoe (Zingiberales: Zingiberaceae) Essential Oil

Zingiber officinale roots were bought from the local market, washed carefully and dried at 40 °C. Dried roots were ground with a grinder. The essential oil was extracted by steam distillation method using Clevenger Apparatus. The essential oils obtained were placed in Eppendorf tubes and kept in the freezer at -20°C until used in the experiment.

Experiment of *Zingiber officinale* Essential Oil on *Acanthoscelides obtectus* under Laboratory Conditions

Contact effect dose experiment

The pure essential oil obtained from *Z. officinale* will be considered as a stock solution and the doses were determined by dilution there. Accordingly, ethyl alcohol was used as a solvent to prepare 100%, 50%, 25% and 12.5% concentrations. Each concentration, 1 µl/insect for each insect, was applied dorsal to the abdomen of the insect by using a micropipette. The experiment was set up in a randomized parcel design and conducted with 5 replicates and 2 repeated, and each replicate had 10 insects. Approximately 1 g of beans were washed, dried, and then placed in a 105cc glass jar. The dose applied insects were placed in them. The glass jars were covered with muslin with the use of rubber bands. They were kept in an incubator at 27±2°C and 60%. Experiments were counted at 24th, 48th, 72nd and 96th hour and dead and live individuals were recorded.

Fumigant effect dose experiment

The pure essential oil obtained from *Z. officinale* will be considered as a stock solution and the doses were determined by dilution there. Accordingly, ethyl alcohol was used as a solvent to prepare 100%, 50%, 25% and 12.5% concentrations. 2cm*2cm sterilised filter paper was glued to the lids of 105 cc glass jar. For each glass jar, 5 µl, 10 µl, 20 µl and 30 µl of essential oils were applied with micropipette. 10 insects were added in glass jar and then each lid was seal strip carefully. The experiment was set up in a randomized parcel design and conducted with 5 replicates and 2 repeated. They were kept in an incubator at 27±2°C and 60%. Experiments were counted at 24th and 48th hour and dead and live individuals were recorded.

Assessment of Data

LC_{50} and LC_{90} values were calculated using POLO-PC (LEORA Software, 1994) package programme. In the experiment, % mortality rates of insects were calculated according to Abbott formula and analysed by variance analysis and Duncan multiple comparison test was applied.

RESULTS

Zingiber officinale Essential Oil Content

Zingiber officinale content is given in Table 1. (Lawrence, 1995).

Table 1. Components of the Essential Oil of *Zingiber officinale*.

Components	**Concentration (%)**
Zingiberene	38.1%
ar-Curcumene	17.1%
β-Sesquiphellandrene	7.2%
Camphene	4.7%
β-Bisabolene	5.2%
β-Phellandrene	2.5%
Borneol	2.2%
1,8-Cineole	2.1%
α-Pinene	1.3%
β-Elemene	1.2%

Contact effect of *Zingiber officinale* essential oil against *Acanthoscelides obtectus*

In the experiment to determine the contact effect of *Z.officinale* essential oil, the highest concentration was accepted as 100% pure essential oil and diluted with ethyl alcohol to obtain concentrations of 50%, 25% and 12.5%. Ethyl alcohol was used for dilution in the control.

The contact activity of *Z.officinale* essential oil against *A.obtectus* was determined by observation at 24th, 48th, 72nd and 96th hour. As a result of the study, it was determined that the mortality effect of the essential oil was found to be quite high. An increase in mortality rates was also observed depending on concentration and time (Figure 1.).

At 24th hour, the highest mortality rate was found at 100% concentration with 100%±0.00. This was followed by 50% concentration (95%±2.24) and 25% concentration (86%±3.05), respectively. At the lowest concentration of 12.5%, the mortality rate was 53%±2.13 at the end of 24th hour (Figure 1.). The difference between the concentrations was found to be statistically significant ($F_{(4,45)}$ =457,86 $p<0.05$). At 48th hour, the highest mortality rate was found at 100% and 50% concentration with 100%±0.00. This was followed by 25% concentration (91%±3.15) and 12.5% concentration (71%±3.15), respectively. The difference between the concentrations was found to be statistically significant ($F_{(4,45)}$ =449.57 $p<0.05$) (Figure 1.). At 72nd hour, the highest mortality rate was found at 100% and 50% concentration with 100%±0.00. This was followed by 25% concentration (96%±1.63) and 12.5% concentration (82%±3.27), respectively. The difference between the concentrations was found to be statistically significant ($F_{(4,45)}$ =690.30 $p<0.05$) (Figure 1.). At 96th hour the highest effect was observed. At the lowest concentration of 12.5%, the mortality rate was found to be 95%±2.24. The mortality rate was observed to be 98%±1.33 at 25% concentration and 100%±0.00 at 100% and 50% concentrations. The difference between the concentrations was found to be statistically significant ($F_{(4,45)}$ =1427,31 $p<0.05$) (Figure 1.).

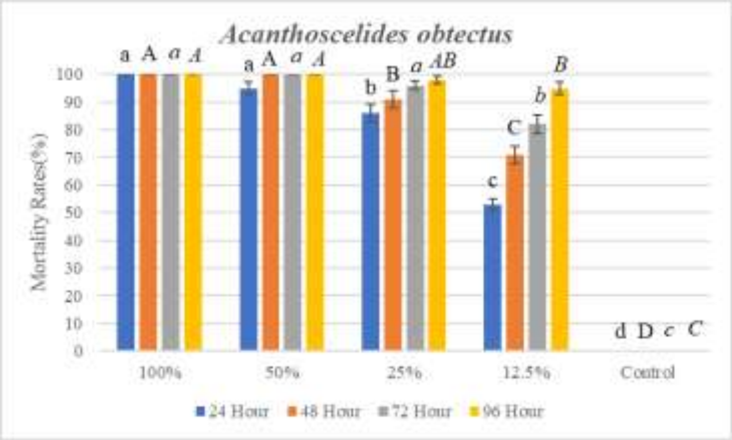

Figure 1. Contact effect of *Zingiber officinale* essential oil against *Acanthoscelides obtectus*

LC_{50} and LC_{90} values were calculated because of the experiment to evaluate the contact effect of *Z. officinale* essential oil against *A.obtectus.* (Table 2.)

As a result of the data found, LC_{50} value was 11.50 at the end of 24 th hour, 8.65 at the end of 48 th hour and 6.49 at the end of 72 nd hour. LC_{90} value was 32.07 at the end of 24 th hour, 21.65 at the end of 48 th hour, and 16.56 at the end of 72 nd hour. At the end of the 96th hour, LC_{50} and LC_{90} values could not be calculated since mortality over 95% was determined.

Table 2. LC_{50} and LC_{90} values obtained because of the experiment to determine the contact effect of *Zingiber officinale* against *Acanthoscelides obtectus*

Times	LC_{50}	LC_{90}	Slope	Chi-Square	Df	Heterogeneity
24 Hour	11.50 (8.99-13.64)	32.07 (27.38-39.99)	2.88±0.37	23.54	38	0.62
48 Hour	8.65 (5.84-10.72)	21.65 (18.62-26.88)	3.22±0.56	19.57	38	0.52
72 Hour	6.49 (2.94-8.90)	16.56 (13.75-20.49)	3.15±0.74	14.46	38	0.38
96 Hour	*	*	*	*	*	*

(* LC_{50} and LC_{90} values were not calculated)

Fumigant Effect of *Zingiber officinale* Essential Oil Against *Acanthoscelides obtectus*

In this study, fumigant activity of *Z.officinale* essential oil against *A.obtectus* was evaluated. In this experiment, 100%, 50%, 25% and 12.5% concentrations were used in 5µl, 10µl, 20µl and 30µl doses. Ethyl alcohol used in dilution was used as control.

The highest mortality rate at the end of 24th hour was 14%±3.71 at 100% concentration because of the application of 5 µl dose of *Z.officinale* essential oil. This was followed by 50% concentration with 13%±3.96 and 25% concentration with 6%±2.21 respectively.

The difference between the concentrations was found to be statistically significant ($F_{(4,45)} = 6.67$ $p<0.05$) (Figure 2.). At 48th hour, the highest mortality rate was found in 100% concentration with 17%±3.96. This was followed by 50% concentration with 15%±3.73 and 25% concentration with 9% ± 2.33, respectively ($F_{(4,45)} = 9.24$ $p<0.05$).

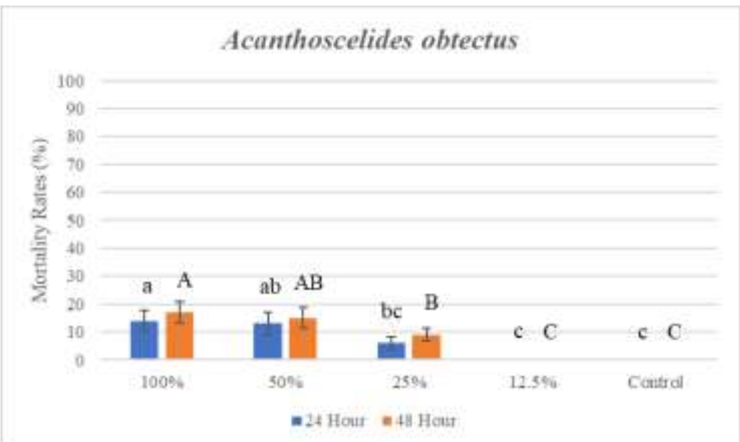

Figure 2. Fumigant effect of 5 µl of *Zingiber officinale* essential oil against *Acanthoscelides obtectus*

LC_{50} and LC_{90} values were determined because of the experiment to determine the fumigant effect against *A.obtectus* by applying 5 µl of *Zingiber officinale* essential oil (Table 3.).

As a result of the data found, LC_{50} value was 558.28 at the end of 24^{th} hour, 464.77 at the end of 48^{th} hour. LC_{90} value was 5804.92 at the end of 24^{th} hour, 5136.12 at the end of 48^{th} hour (Table 3.).

Table 3. LC_{50} and LC_{90} values were obtained because of the experiment to determine the fumigant effect of *Zingiber officinale* essential oil applying 5 µl against *Acanthoscelides obtectus.*

Times	LC_{50}	LC_{90}	Slope	Chi-Square	Df	Heterogeneity
24 Hour	558.28 (230.31-9236.54)	5804.92 (1085.37-1384690.87)	1.26± 0.32	40.49	38	1.07
48 Hour	464.77 (214.96-3654.58)	5136.12 (1127.27-337510.79)	1.23±0.30	35.72	38	0.94

The highest mortality rate was determined at the end of 24^{th} hour because of the application of 10 µl dose of *Z. officinale* essential oil was 16%±2.67 at 100% concentration. This was followed by 50% and 25% concentration with 9% ±2.33. The difference between the concentrations was found to be statistically significant ($F_{(4,45)}=10.29$ $p<0.05$) (Figure 3.).

At 48^{th} hour, the highest mortality rate was found at 100% concentration with 20%±2.98. This was followed by 50% concentration with 14%±3.05 and 25% concentration with 13%±2.60, respectively. The difference between the concentrations was found to be statistically significant ($F_{(4,45)}=13.48$ $p<0.05$) (Figure 3.).

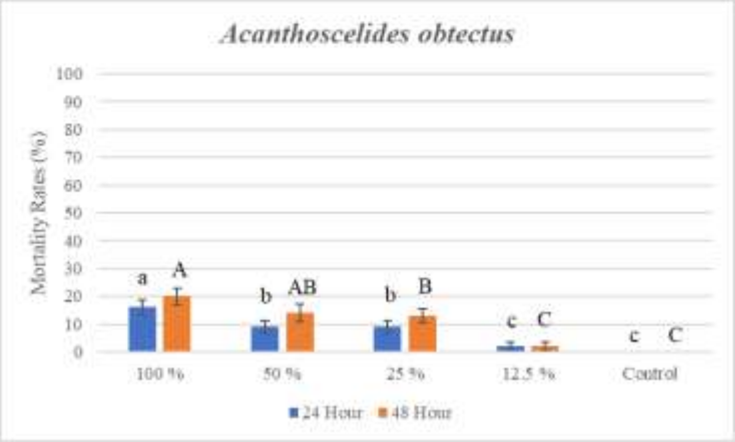

Figure 3. Fumigant effect of 10 µl of *Zingiber officinale* essential oil against *Acanthoscelides obtectus*

LC_{50} and LC_{90} values were determined because of the experiment to determine the fumigant effect against *A.obtectus* by applying 10 µl of *Z.officinale* essential oil (Table 4.).

As a result of the data found, LC_{50} value was 1153.60 at the end of 24th hour, 624.24 at the end of 48th hour. LC_{90} value was 27684.66 at the end of 24th hour, 12553.79 at the end of 48 th hour (Table 4.).

Table 4. LC_{50} and LC_{90} values were obtained because of the experiment to determine the fumigant effect of *Zingiber officinale* essential oil applying 10 µl against *Acanthoscelides obtectus.*

Times	LC_{50}	LC_{90}	Slope	Chi-Square	Df	Heterogeneity
24 Hour	1153.60 (325.69-166306.75)	27684.66 (2420.32-495368254.86)	0.93± 0.29	25.66	38	0.68
48 Hour	624.24 (239.40-12028.74)	12553.79 (1761.89-6451792.19)	0.98± 0.26	27.55	38	0.73

The highest mortality rate was determined at the end of 24th hour because of the application of 20 µl dose of *Z. officinale* essential oil was 39%±3.15 at 100% concentration. This was followed by 50% concentrations with 28%±3.26 and 25% concentration with 20%±2.11. The difference between the concentrations was found to be statistically significant ($F_{(4,45)}$ =43.22 $p<0.05$) (Figure 4.).

At 48th hour, the highest mortality rate was found at 100% concentration with 50%±4.71. This was followed by 50% concentration with 34%±2.67 and 25% concentration with 27%±3.00, respectively. The difference between the concentrations was found to be statistically significant ($F_{(4,45)}$ =37.89 $p<0.05$) (Figure 4.).

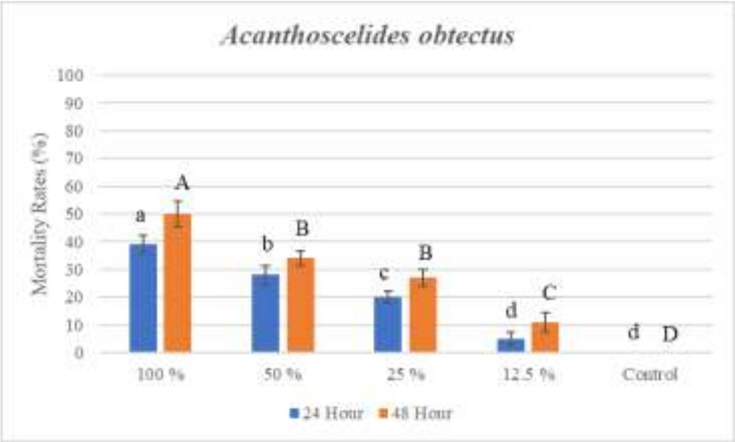

Figure 4. Fumigant effect of 20 µl of *Zingiber officinale* essential oil against *Acanthoscelides obtectus*

LC_{50} and LC_{90} values were determined because of the experiment to determine the fumigant effect against *A.obtectus* by applying 20 µl of *Z. officinale* essential oil (Table 5.).

As a result of the data found, LC_{50} value was 146.07 at the end of 24th hour, 98.27 at the end of 48th hour. LC_{90} value was 1366.4 at the end of 24th hour, 1035.0 at the end of 48 th hour (Table 5.).

Table 5. LC_{50} and LC_{90} values were obtained because of the experiment to determine the fumigant effect of *Zingiber officinale* essential oil applying 20 µl against *Acanthoscelides obtectus.*

Times	LC_{50}	LC_{90}	Slope	Chi-Square	Df	Heterogeneity
24 Saat	146.07 (100.27-291.44)	1366.4 (556.32-8200.45)	1.32±0.23	20.70	38	0.55
48 Saat	98.27 (72.053-166.11)	1035.0 (451.63-5184.98)	1.25±0.21	26.48	38	0.70

The highest mortality rate was determined at the end of 24th hour because of the application of 30 µl dose of *Z. officinale* essential oil was 41%±4.33 at 100% concentration. This was followed by 50% concentrations with 25%±3.15 and 25% concentration with 23%±30. The difference between the concentrations was found to be statistically significant ($F_{(4,45)}$ =30.48 $p<0.05$) (Figure 5.).

At 48th hour, the highest mortality rate was found at 100% concentration with 97%±2.13. This was followed by 50% concentration with 71%±3.48 and 25% concentration with 41%±5.71 respectively. The difference between the concentrations was found to be statistically significant ($F_{(4,45)}$ =64.63 $p<0.05$) (Figure 5.). The results showed that the highest mortality effect was determined at 100% concentration in both time periods (Figure 5.).

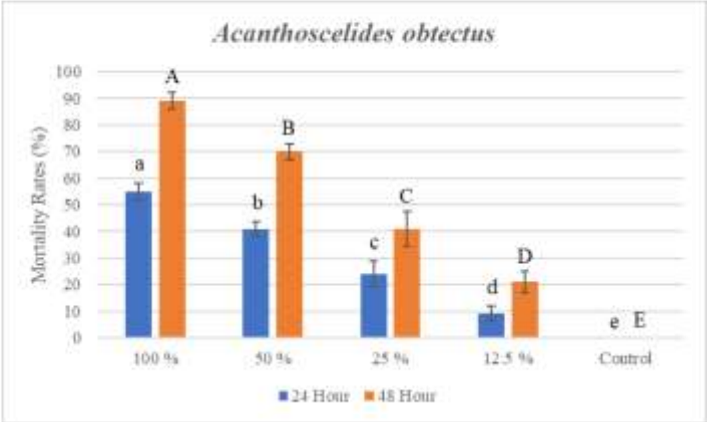

Figure 5. Fumigant effect of 30 µl of *Zingiber officinale* essential oil against *Acanthoscelides obtectus*

LC_{50} and LC_{90} values were determined because of the experiment to determine the fumigant effect against *A.obtectus* by applying 30 µl of *Z. officinale* essential oil (Table 6.).

As a result of the data found, LC_{50} value was 76.48 at the end of 24^{th} hour, 29.65 at the end of 48^{th} hour. LC_{90} value was 497.30 at the end of 24^{th} hour, 108.49 at the end of 48^{th} hour (Table 6.).

Table 6. LC_{50} and LC_{90} values were obtained because of the experiment to determine the fumigant effect of *Zingiber officinale* essential oil applying 30 µl against *Acanthoscelides obtectus.*

Times	LC_{50}	LC_{90}	Slope	Chi-Square	Df	Heterogeneity
24 Hour	76.48 (61.18-105.14)	497.30 (285.74-1271.40)	1.58± 0.22	26.36	38	0.69
48 Hour	29.65 (25.61-34.04)	108.49 (86.12-149.79)	2.275±0.22	37.35	38	0.98

DISCUSSION AND CONCLUSION

Legumes are cheap and important agricultural products in terms of contained nutrients. It is consumed both fresh and dried and is an important raw material for the food industry. In recent years, people who do not include meat protein in their diet have supplied this protein from legumes. For this reason, there is an increase in the importance of legume consumption.

Legumes, which are an important agricultural product in our country, are cultivated in almost all regions. After the harvest, the products are stored until they go to the final consumer. It is also necessary that the quantity and quality of the product do not change while storage. One of the most important factors affecting the quality and quantity of the product is pests. *Acanthoscelides obtectus* is the most important pest in legumes. Therefore, chemicals are increasingly used in the control of these pests. The intensive use of chemicals leads to resistance and has negative effects on non-target organisms. Therefore, researchers have focused on alternative methods to suppress these pests.

In this study, contact and fumigant activities of *Z. officinale* essential oil against *A.obtectus* were evaluated. In the fumigant effect experiment, the highest mortality rates were obtained at the end of 48th hour in 30 µl applications of *Z.officinale* essential oil to *A.obtectus*. At a dose of 100%, 89 % mortality was recorded. It was found that there were increases in mortality rates from low dose to high dose and depending on time and these increases were determined to be statistically significant. Kim et al. (2003) examined the contact and fumigant activity of methanol extracts and 5 essential oils of 30 medicinal and aromatic plants against *Callosobruchus chinensis* and showed that the essential oils were effective. In the experiment in which the contact effect of essential oil was found, the effectiveness of 12.5%, 25%, 50% and 100% doses against *A.obtectus* was determined at the end of 24th , 48th , 72nd and 96th hour and it was found that the mortality rates increased depending on the dose and time and this increase was statistically significant. The lowest mortality rate of 53% was found at a concentration of 12.5% at the end of 24th hour. At a dose of 100%, 100% mortality was observed after 24th hour. This was followed by mortality rates of 95% at 50% dose and 86% at 25% dose.

The results showed that the essential oil obtained from *Z.officinale* showed fumigant and insecticidal activity against *A.obtectus*. It is important to evaluate these formulations in the control of other storage pests. The results to be obtained will provide a significant contribution to the application of this formulation in practice.

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REFERENCES

Ahmad, I., Hasan, M., Arshad, M., Khan, M., Rehman, H., Zahid, S., Arshad, M., 2016. Efficacy of different medicinal plant extracts against *Rhyzopertha dominica* (Fabr.)(Bostrichidae: Coleoptera). Journal of Entomology and Zoology Studies 4, 87-91

Alkan, M., Gökçe, A. 2012. *Tanacetum abrotanifolium* (L.) Druce (Asteraceae)'un gövde ve çiçek ekstraktlarının *Sitophilus granarius* ve *Sitophilus oryzae* (Col.,Curculionidae)'ye olan kontakt ve davranışsal etkileri. Türkiye Entomoloji Dergisi, 36 (3), 377-389

Anonymous, 2008. Zirai Mücadele Teknik Talimatları Cilt:1 Tarım ve Köy işleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü Bitki Sağlığı Araştırmaları Daire Başkanlığı, Ankara 283s.

Bayar, F. U. 2020. Doğadan gelen mucize: Zencefil (*Zingiber officinale*). Bahçe, 49(2), 99-110.

Burçak, A.A., Duru, A.U., Örnek H., 2015.Bitki Koruma Ürünleri ve Pestisit Kalıntıları. T.C. Gıda, Tarım ve Hayvancılık Bakanlığı Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü Bitki Sağlığı Araştırmaları Daire Başkanlığı. 187s.

Çetin, H., Uysal, M., Şahbaz, A., Alaoğlu, Ö., Akgül, A., Özcan, M. M.2015. Tıbbi ve Aromatik Bitki Uçucu Yağlarının Fasulye Tohum Böceği [*Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae)] Erginlerine Fumigant Etkileri. Selçuk Tarım Bilimleri Dergisi, 1(1), 6-11.

Dimetry N.Z. 2014. Different plant families as bioresource for pesticides. 1-20pp. In.Singh, D. (Ed.). . Advances in plant biopesticides. Springer.

Dizlek, H. 2012. Tahılların depolanmasında etkili olan başlıca etmenler. Gıda Teknolojileri Elektronik Dergisi, 7(2), 48-59.

Ertürk S., Ahmet G.F., Mevlüt E., 2017. Evaluation of diatomaceous earth formulations for the control of rice weevil, *Sitophilus oryzae* L.,1763 (Coleoptera: Curculionidae) in stored rice Türk.entomol.derg., 41(3):347-354

Kaplan, H., 2005. Zencefilin (*Zingiber officinale* Roscoe) bitkisel özellikleri ve yetiştiriciliği. Derim Dergisi, 22(2).

Karakoç Ö.C., Gökçe A., Telci İ. 2006.Bazı bitki uçucu yağlarının *Sitophilus oryzae* L., *Sitophilus granarius* L. (Col.,Curculionidae) ve

Acanthoscelides obtectus Say. (Col: Bruchidae)'a karşı fumigant etkileri Türk.Entomol.Derg.,2006,30(2):123-135

Karakoç Ö, C.., Tüfekçi A.R., Demirtaş İ., İpek A. 2013. *Salvia tchitatheffi* ve *Salvia cryptantha* Uçucu Yağlarının ve Ekstraklarının İki Önemli Depo Zararlısı Üzerindeki İnsektisidal Aktiviteleri Tarım Bilimleri Araştırma Dergisi 6(1) :155- 158,

Kim, S. I., Roh, J. Y., Kim, D. H., Lee, H. S., Ahn, Y. J. 2003. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis.* Journal of Stored products research, 39(3), 293-303.

Lawrence, B.M., 1995. Progress in essential oil. Perfume & Flavorist 20.

Loni, A., Panahi, O. 2015. Control of stored grain pest, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), using the essential oils isolated from *Zingiber officinale* (L.) and *Mentha pulegium* (L.) in laboratory condition. Archives of Phytopathology and Plant Protection, *48*(5), 434-440.

Mohammadi Nori, H., Shakarami, J., Jafari, S. 2018. Ovicidal and larvicidal activity of nine botanical powders against *Callosobruchus maculatus* F. (Col: Bruchidae). IAU Entomological Research Journal, 9(4), 359-371.

Regnault-Roger, C., Hamraoui, A., Holeman, M. et al., 1993. Insecticidal effect of essential oils from mediterranean plants upon *Acanthoscelides obtectus* Say (Coleoptera, Bruchidae), a pest of kidney bean (*Phaseolus vulgaris* L.). J Chem Ecol 19, 1233–1244 https://doi.org/10.1007/BF00987383

Suthisut, D., Fields, P.G., Chandrapatya, A., 2011. Fumigant toxicity of essential oils from three Thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids. Journal of Stored Products Research 47 (3), 222-230.

CHAPTER 15

EXAMINING THE SUPPLY CHAIN MANAGEMENT OF DAIRY ENTERPRISES AND EVALUATING FACTORS INFLUENCING SUPPLIER SELECTION

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1. INTRODUCTION

The importance of nutrition in maintaining human health is undeniable. Adequate and balanced nutrition is defined as the daily intake of appropriate amounts of nutrients such as energy, protein, and other essential elements that the body requires. These essential nutrients, which are frequently needed in daily life, are provided through foods such as meat, milk, vegetables, fruits, and grains. Milk, as an animal-derived food, is considered a significant dietary component that should be consumed throughout all stages of life due to its content of vitamin C, iron, and various micro and macro nutrients (Ünal and Besler, 2012:7; Çelik, 2002:44).

When examining milk production and the number of milking animals in Turkey, it is observed that the quantity of raw milk production in 2022 decreased by 7.1% compared to the previous year, parallel to declines in livestock numbers and productivity. In 2021, raw milk production was 23.2 million tons, whereas it decreased to 21.6 million tons in 2022. Of the total raw milk production, 92.3% comes from cow milk, 7.5% from sheep and goat milk, and 0.2% from buffalo milk. In 2022, sheep and goat milk production decreased by 8.9% to 1.6 million tons, cow milk production decreased by 6.8% to 19.9 million tons, and buffalo milk production decreased by 31.5% to 43.6 thousand tons. In 2023, there was minimal change with a total of 21.5 million tons of raw milk produced, including 1.5 million tons of sheep and goat milk, 19.9 million tons of cow milk, and 43.1 thousand tons of buffalo milk (TUIK, 2023). Another reason for the decline in milk production is the increasing production costs in the sector (Ataseven, 2023). Rising input costs such as feed, energy, labor, and veterinary services have negatively affected dairy farms, reducing the productivity of producers who have had to downsize their livestock.

In today's global economy, achieving competitive advantage and sustaining performance for businesses depend heavily on effective supply chain management. In this context, supply chain management and supplier selection hold strategic importance, especially in the dairy industry. Dairy enterprises must develop an effective supply chain management strategy not only to deliver quality products but also to reduce costs, ensure timely delivery, and enhance customer satisfaction. Supplier selection and supply chain management play a crucial role in enabling dairy enterprises to operate efficiently. The concept of supply chain has evolved into a long-term partnership rather than a short-term buyer-supplier relationship (Güler and Saner, 2020:207). Therefore, identifying and evaluating factors that influence the selection of supplier partnerships is crucial for business owners. Choosing the right supplier can significantly impact cost reduction and contribute to the sustainability of the enterprise.

In recent years, the continuity of animal production is at risk due to increasing input costs coupled with the inability of produced goods to achieve market value. The adverse effects of applied import policies on producers should also not be overlooked. Strategies and methods that can enhance the efficiency and productivity of enterprises engaged in animal production activities are crucial at this stage. The role of suppliers is significant in enabling these enterprises to sustain their operations and conduct milk production. By implementing an effective supply chain, businesses can reduce costs and increase profitability. In this context, the primary aim of this study is to examine the supply chain management practices of enterprises involved in milk production and evaluate the factors influencing supplier selection. Analyses conducted in critical areas such as supplier relationship management, logistics management, and inventory management will assist enterprises in making strategic decisions. While literature review reveals studies addressing supplier selection in various sectors, there is a noticeable scarcity of research specifically focusing on supplier selection for dairy producers. Hence, this study aims to contribute to the existing literature in this field as another objective.

2. CONCEPTUAL FRAMEWORK

Initially, the topic of supply chain and its management is discussed, and then the focus shifts to examining supply chain management within the operations of dairy companies.

2.1. Supply Chain Management

The concept of the supply chain first emerged in J.B. Houlihan's 1985 publication, where he combined strategic business decisions with a logistics focus, treating the supply chain as a single entity. Supply Chain Management (SCM) is a model that involves the systematic control and coordination of inter-firm processes to reduce costs, enhance quality, and increase the speed of operations (Özdemir, 2004:87). A supply chain is a network structure consisting of service and distribution options that perform material and raw material procurement processes, convert them into semi-finished and finished products, and then deliver them to customers through distribution channels (Eymen, 2007:7).

In managing the supply chain, the goal is to effectively integrate subcontractors, production facilities, warehouses, and sales points to ensure that goods are available at the right time, in the right place, and in the right quantity to meet customer needs, while reducing the total system cost (Büyüközkan and Vardaloğlu, 2008:4). The primary priority in supply chain management is to deliver the product to the buyer as quickly as possible without deterioration, thereby maintaining its commercial value (Çancı and Erdal, 2013 as cited in Bircan, 2016:2). Additionally, SCM aims to increase customer satisfaction, reduce operational costs, product defects, inventory-related costs, and cycle time (Özdemir, 2004:89).

A supply chain can be defined as the sum of all direct and indirect processes necessary to fulfill a company's order. Supply chain management, on the other hand, is the process of managing the flow of resources and information to ensure that a product reaches the right consumer at the right time, at an appropriate price, and with low-cost expenses from the business perspective (Erkara, 2021:62). The supply chain should not be perceived merely as involving the producer and supplier; it encompasses a broader scope that includes transporters, wholesalers, customers, and retailers. It is characterized as a network comprising business stages, including sourcing raw materials, procuring materials, manufacturing production using these raw materials and materials, storing products based on production results, and directing them to customers and retailers. The purpose of this network is to manage the strategic stages of businesses efficiently and effectively and to consider it as a supply chain management process (Turan et al., 2012:59). Supply chain processes begin with the development of new products and include marketing, production, distribution, financing, and after-sales services.

Success in dairy businesses can be achieved through the proper implementation of these supply chain processes with the right strategies. From the perspective of dairy businesses, effectively organizing and operating supply chain management plays a critical role in increasing profitability and sustainability. The literature review reveals that there are limited studies examining the supply chain structure of dairy businesses. One such study was conducted by Güler and Saner in 2020. Their research aimed to determine the feed supplier and milk buyer preferences of dairy farming enterprises. They collected data through face-to-face surveys with producers in İzmir and Manisa, evaluating feed suppliers based on quality, price, and payment flexibility criteria, and milk buyers based on price, payment duration, and milk purchase quantity. The study concluded that quality is a critical factor in feed supply, while purchase quantity is an influential factor in evaluating milk buyers. Another study by Deste and Yurttaş (2018) aimed to identify the criteria and their priorities that businesses in the dairy sector should consider in the supplier evaluation process. Businesses in the Southeastern Anatolia region were included in the research, and the collected data were grouped under the headings of price, quality, hygiene, and service using the nominal group technique.

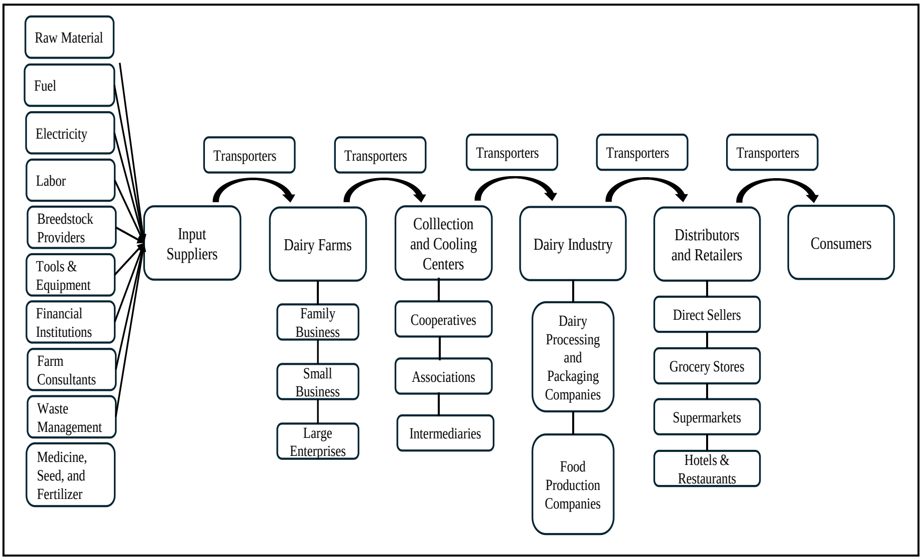
These studies highlight the importance of well-structured supply chain management in the dairy industry. Effective supply chain management ensures that dairy businesses can respond swiftly to market demands, maintaining the quality and freshness of their products. By focusing on key factors such as quality, price, and logistics, dairy enterprises can optimize their operations and improve customer satisfaction. Moreover, continuous evaluation and improvement of supply chain processes can lead to long-term sustainability and competitiveness in the market. Thus, adopting innovative SCM practices is essential for dairy businesses aiming to thrive in a dynamic and challenging environment.

2.2. Dairy Supply Chain Management

The supply chain of dairy farms exhibits distinct characteristics from other supply chains. Preserving product freshness and adhering to hygiene conditions are crucial requirements for dairy farms. The dairy supply chain encompasses complex processes including the production of raw milk, its transformation into dairy products, transportation, processing, storage, packaging, and finally delivery to consumers (Anggrahini, Baihaqi, and Anggani, 2018:2918).

Figure 1 illustrates the supply chain structure of dairy farms. In the supply chain management process of dairy farms, there are input-providing suppliers, dairy farms themselves, collection and cooling centers, transporters, wholesalers, dairy processing companies (dairy industry), retailers, and consumers (Güler and Saner, 2017:166; Van der Lee, Bebe, and Oosting, 2016:19). This chain structure involves various relationships at each stage, including the establishment of necessary infrastructure for milk production, animal procurement and feeding processes, procurement of milk from farms, transportation to factory premises, and distribution through channels to reach consumers.

Figure 1. Supply Chain Structure of Dairy Enterprises



Source: Compiled from Van der Lee, Bebe, Oosting (2016) and Güler & Saner (2017) studies, adapted by the researcher for the purpose.

In dairy farms, meeting the needs of existing animals by providing them with a welfare-oriented, healthy feeding environment is a fundamental goal. Therefore, it is essential in the dairy farm supply chain to ensure the procurement of inputs. Identifying the correct and suitable suppliers for these inputs can help reduce the costs of dairy farms. Moreover, achieving high-quality products (with high protein and fat content, suitable color, odor, etc.) can provide a price advantage in the market.

3. RESEARCH METHOD

In this stage of the study, factors determining supplier selection in dairy farms were identified through literature review and field research findings. The findings were evaluated specifically in the context of dairy farms.

For the literature review, researches related to dairy farms were primarily reviewed. Key terms (dairy farms, supplier selection, economic analysis of dairy farms) were used to search national and international academic journals and national thesis databases.

In addition to secondary data, primary data was collected through field research. The conducted study is qualitative in nature. Qualitative research is generally conducted with a purposive sample of a small number of cases. Qualitative samples can sometimes include a single individual or a small group (Strauss and Corbin, 1998). Determining the sample size is often about balancing breadth and depth and reaching saturation point where no new information is obtained (Baltacı, 2018:261). Therefore, in the conducted fieldwork, a specific participant limit was not set, and the study was concluded once similar statements and findings were identified. A total of 38 interviews were conducted, and the collected data was evaluated.

The interviews were conducted face-to-face with farmers using a semi-structured interview form in the central district of Kırşehir province and Ortaköy district of Aksaray province. Farmers were asked about their suppliers, criteria they consider important in supplier selection, and other topics such as waste management practices, energy supply methods, and labor issues at their farms.

3.1. Findings and Discussion

First of all, findings regarding suppliers providing inputs and forming the beginning of the supply chain management process for dairy farms have been presented and evaluated. Various studies on the economic profitability and efficiency analyses of dairy farms (Uyanık, 2000; Günlü and Sakarya, 2001; Şahin et al., 2001; Bayramoğlu, 2003; İçöz, 2004; Nizam and Armağan, 2006; Tandoğan, 2006; Gündüz and Dağdeviren, 2011; Keskin and Dellal, 2011; Murat and Sakarya, 2012; Çelik and Sarıözkan, 2017; Yılmaz et al., 2019; Bulut and Paksoy, 2023) reveal that raw material expenses constitute the largest share of variable costs. Subsequently, veterinary services, fuel expenditures, temporary labor costs, transportation expenses, electricity costs, machinery and equipment costs, and financial service expenses are significant expenditures for dairy farms, each considered as suppliers providing inputs to the operations. Dairy farms evaluate each supplier using selection criteria and appropriate metrics to ensure their demands are consistently met at competitive prices, at the right place and time, in the right quantity, and with high quality (Deste and Yurttaş, 2018:186). The correct selection of these suppliers, which hold significant positions within the dairy farm's supply chain structure, directly affects the operational efficiency of the enterprise.

Examining the literature on supplier selection (Candan and Yazgan, 2015; Bircan, 2016; Ecer and Küçük, 2008; Kapar, 2013; Altuntaş and Özdemir, 2021; Beil, 2010; De Boer, 2001; Echefaj, 2023), it is evident that common factors such as price, quality, service, and delivery are frequently used in their evaluation. However, each sector may have its own internal dynamics, leading to the addition of other factors to these primary criteria.

By integrating the findings from fieldwork with the literature on dairy farms and supplier selection, the factors influencing the selection of suppliers providing inputs to dairy farms have been separately evaluated for each supplier.

In the context of variable expenses, raw material costs hold the highest share. When determining the supplier for roughage and concentrated feed delivery, factors such as feed price, followed by quality, payment terms, payment flexibility, reliability, delivery method, product wastage, product returns, customer services, after-sales support, ration support, and feed analysis support are considered (Altuntaş and Özdemir, 2021:287). Therefore, these factors identified in dairy farms are considered crucial in supplier selection.

Regarding fuel, it is used during the stage of producing feed crops for dairy farms (Nizam and Armağan, 2006:55) and in the process of preparing feed for animals. This expense item increases parallel to the number of animals. Another reason for the increase in fuel expenditure is the quality of the fuel itself. The fuel used is "diesel." Also known as mazot, this fuel is obtained as a primary product while boiling oil at temperatures ranging from 200-300 degrees. Differences in diesel prices at stations can be considered as an indicator of the fuel's quality. However, the most important criterion determining the quality of diesel is the cetane number. High-quality diesel results in extended tractor usage, fewer breakdowns, and prevents oxidation and decay of the diesel tank, thereby reducing operational costs for farms. Fuel price is the foremost factor in supplier selection for dairy farms, followed by service quality, product quality, and payment ease (Görçün, 2020:30). Evaluated from the perspective of dairy farms, besides having a low price, preference is given to a familiar and reliable gas station. As a result, product and service quality will increase. Savings will be made in the use of inputs, and maintenance costs related to possible tractors and other equipment will be reduced.

Electricity suppliers do not have an alternative product that can be replaced due to the monopoly market. However, dairy farms may prefer renewable energy investments based on their current electricity expenses to reduce their operating costs in the long term.

Regarding labor supply, the basic approach of producers is to employ skilled individuals who are proficient in both animal care and crop production processes, who know and understand agricultural tools and equipment. Expanding employment volume can rectify income distribution in dairy farms, surpassing the market's institutional structure in terms of the method of supplying labor through traditional methods.

Breeding suppliers are critical suppliers for the sustainable production of dairy farms. It is expected that these reliable suppliers will provide rapid delivery and supply breeding materials at reasonable prices to ensure the sustainability of operations.

In their study aimed at identifying the supplier of planting machines and equipment, Nedeljković, Puška, and Đokić (2022:229) found that factors such as company reputation, payment flexibility, product quality, covering transportation costs, distance to the farm, and the use of technology and innovation determine the supplier of equipment. From the perspective of dairy farms, the use of modern equipment and machinery has increased in recent years, transitioning towards technology-intensive production processes. According to interviews, producers predominantly use milking machines, feed preparation and mixing machines, cleaning devices for hygiene, and cooling tank equipment in their operations. Particularly, having a milking system instead of just a milking machine saves time for farms. However, this is not feasible in every dairy farm. The primary features dairy farms seek in their equipment suppliers are post-sales services, as there is little differentiation among the products offered by service companies in the sector. Product quality, payment ease, and innovation in product introduction can be considered other factors.

In financial institutions, studies (Bilge, 2018) show that factors affecting the choice of financial institution include the bank's convenient location, being a state bank, low transaction costs, easy access to credit, and confidentiality of personal information. The state's provision of low-interest credit support allows businesses to benefit from these credits, receive diesel-fertilizer support, renew equipment, and extend the delivery period of purchased raw materials, resulting in increased confidence in dairy farms. In interviews with producers, it was found that the factors that determine their choice of financial institutions are the ease of using credit and the presence of a state bank for both trust and cost-effectiveness. Therefore, it can be evaluated that dairy farms should prioritize factors such as ease of credit use, cost-effectiveness, and the presence of a state bank in determining their financial institution preferences.

Producers need information to improve their farm's competitiveness and product quality, and to minimize negative factors such as waste rates and animal diseases. Given that milk production is a specialized process influenced by various dynamics, producers involved in this work need to increase their knowledge level and develop their skills by employing farm consultants. Research indicates various factors influencing the choice of farm consultant, namely the content of consultation, consultation technique, and duration (Tamuliene, Raupeliene, and Kazlauskiene, 2017:79). In dairy farms, consulting on controlling feed quality, followed by ration preparation, hygiene conditions, product storage, and logistics, stands as a significant area of consultancy. Therefore, dairy farms require consultants capable of responding promptly and accurately to these issues under suitable conditions, ultimately resulting in increased milk yield and animal health protection.

Waste management is a crucial aspect that must be controlled in livestock farming operations from the perspectives of both business, environmental, and economic considerations. Waste management on livestock farms involves the collection, removal, accumulation, and storage of solid and liquid wastes produced in shelters, followed by their maturation and application to fields as farm manure. The wastes generated in operations are processable and controllable wastes; however, their uncontrolled disposal can harm natural resources, spread unpleasant odors in the environment, and cause groundwater pollution (Erkan Can and Boğa, 2018:260). Dairy farms require suppliers providing waste management services for the removal of animal waste from the field. In interviews with farm owners, it was understood that they prefer companies with biogas plants for managing waste generated on the farm. The most important factor here is that the contractor firm covers transportation costs. Therefore, the primary factor in selecting suppliers for waste management services in dairy farms is transportation costs. Another factor is the prompt implementation of transportation services, indicating the importance of rapid service.

Suppliers of medicine, seeds, and fertilizers are companies that supply inputs required in the crop production processes of dairy farms. Medicines are used in crop production to eliminate pests. Businesses should take the time to visit their trusted agricultural chemical dealers to find a quick and effective solution to the pests that have infested their products rather than consulting with other producers who have no information on the pesticide or disease. According to the responses given in interviews with producers, the factors influencing the choice of medicine suppliers are that the medicine must be ministry-approved, targeted, and the supplier must be reliable.

Trust in the dealer or company is a primary factor in the selection of seed suppliers. Other determining factors include the brand of the seed, its previous trial in the region, its ability to supply productive and especially drought-resistant varieties.

The fertilizer should meet the mineral and elemental requirements for balanced plant nutrition, while also preserving or enhancing soil fertility without any harmful effects on the environment. It should enable producers to cultivate high-yield, high-quality, and safe products. Therefore, selecting a fertilizer supplier is crucial. Recent scientific studies, such as one conducted by Van Thanh et al. in 2022 among producers in southern Vietnam, have evaluated fertilizer suppliers based on main criteria including cost, environmental sustainability (green adequacy), quality, delivery schedule, and environmental management performance, along with related sub-criteria. The prominence of green adequacy criterion highlights the preference for firms that do not harm the environment and promote sustainability, with quality and cost following as determining factors. Another study by Agrawal et al. (2017) in India identified

19 different criteria for fertilizer supplier selection, emphasizing factors such as company history, technology use, product guarantee, product quality, and timely delivery. When evaluating the preference for suppliers of drugs, seeds, and fertilizers by dairy farms, key factors include trust in the dealer or company, availability of productivity-enhancing types and products, and cost-effectiveness, which are prioritized in these operations.

4. CONCLUSION and RECOMMENDATIONS

There are certain factors observed to be effective in dairy farms and in increasing milk yield. Factors such as milk yield, quality milk production, number of dairy cattle, ensuring milk hygiene, attention to cleanliness, color of the produced milk, animal care, feeding methods with organic products, ensuring sustainable production by providing necessary supports in animal purchase, management and marketing processes, and feed quality are among these important factors. While ensuring these factors, reducing costs by including product and innovation elements in the supply chain management method, increasing profitability rate, competitiveness, market level, market share ratio, customer expectation levels and ensuring the best conditions of time, product, service and design stages are required to enhance the performance process of the businesses. In literature, a scan has been made related to the supply chain management of dairy farms in order to evaluate the factors affecting the selection of suppliers of dairy farms. It has been observed that there are not enough studies in the field. In the existing studies, it is necessary to reduce feed prices for the reduction of the most important input feed expense, to increase the production of roughage and concentrate feed and to expand pasture areas in accordance with geographical regions. Other input expenses of dairy farms include veterinary services, vaccination opportunities for reducing animal diseases, drugs for animals and veterinary expenses, variable expenses are involved in the elements. When we consider the time factor in fuel costs, the success rate in the dairy farm centers also decreases as the distance increases. Because storage and cooling processes are limited in milk products. For this reason, dairy farms must either turn their products into durable products or immediately turn them into the market. Apart from that, electricity expenses, machinery equipment purchases, and operating expenses cover the leading expenses of the enterprise. It should not be confined only to these input expenses.

In order to examine the supply chain management of dairy farms and evaluate the factors influencing supplier selection, interviews were conducted with 38 dairy farms in Kırşehir city center and Ortaköy district of Aksaray at a semi-structured interview level. It was observed that the education levels of the participants ranged from elementary school to middle school graduates. According to the interview form, feed costs were identified as the most important criterion among the input costs in the supply chain method. Dairy farms pay attention to feed characteristics such as price increases when sourcing from cooperatives or factories, focusing on feed quality, production site reliability, and sustainability of production sites in the region. Other input factors such as price, quality, service provided, contract terms, reliability, supply quantity, capacity, delivery time, and profitability ratio were also identified as criteria for supplier selection and what dairy farms look for in suppliers.

During the interviews, it was noted that dairy farms experience labor issues and to prevent this, they require certified shepherds, employees who understand tractor equipment and machinery, and provide solutions that facilitate farm operations with sufficient knowledge and experience. Regarding waste management on farms, they often prefer contracted companies with biogas facilities and use animal manure stored in designated areas to enhance crop yields through natural fertilizer methods, although they mention transportation costs. For both animal and plant production, they use Ministry-approved drugs and prefer suppliers from reputable sources, focusing on goal-oriented approaches.

The effective use of supply selection in dairy farms begins with animal material. Factors such as the age, breed, milk production status of the animal are crucial for ensuring efficiency on the farm. Reducing animal disease and mortality rates, increasing milk production efficiency with optimal profitability, improving soil structure, and selecting suppliers using correct methods are important for reducing farm expenses and ensuring effective supply selection. The misconception that a low number of animals leads to a decrease in milk yield is incorrect. To achieve profitability on the farm, attention must be paid to animal care, crop production, breed, and many other factors.

In dairy farms, variable costs in input shares support policies that lead to increased market competition. Literature studies show that there are not enough research studies in the field of supply chain management in dairy farms. Including supply chain management in product and innovation elements enhances the performance of businesses in activities such as time, cost, and design process. Regarding the time factor, as the distance from provincial centers increases, the success rate in farms decreases because storage and cooling facilities for harvested milk products are limited. Therefore, dairy farms need to quickly market their products or transform them into durable goods.

In dairy farms, important factors affecting milk yield include milk yield, milk quality, number of dairy cattle, as well as care and feeding methods, live material, organizational quality, and feed quality, all of which contribute to increased milk production. However, insufficient capital and low education levels among farm managers hinder farms from achieving desired production levels. To address these issues in dairy farms, it is essential for the Ministry to organize informative and awareness-raising training courses through its provincial organizations. Farm managers should also seek support through cooperatives and associations and strive for more knowledge to achieve high income despite low costs.

It is crucial for farms to prioritize animal health, prefer locations closer to milk collection centers for setting up dairy farms, install milk cooling tanks to maintain marketable product prices, and store milk properly using correct methods to increase revenue. Supply chain management is a long-term and comprehensive process that requires supply chain managers to possess necessary knowledge and experience. This involves elements such as procurement, storage, product planning, inventory management, accounting records, and marketing (Güler and Saner, 2017:169).

Studies indicate that capital constraints are a major hindrance to effective supply chain management. Efficient management of existing capital is crucial for businesses. Another significant factor is the education level of farm owners, which is often at elementary and middle school levels according to literature and field studies. Therefore, it is essential to educate and raise awareness among producers about the importance and benefits of supply chains for their farms.

Effective management of this complex supply chain is crucial for ensuring product quality and safety, directly impacting consumer satisfaction.

Dairy farms need to employ robust monitoring and tracking systems to maintain the integrity of their supply chains. Innovations in cold chain logistics and real-time data analytics can enhance the efficiency and reliability of dairy supply chains. Additionally, establishing strong partnerships with all stakeholders from farmers to retailers is essential for smooth coordination and problem-solving. By focusing on continuous improvement and adapting to technological advancements, dairy farms can become more sustainable and competitive in the market.

Livestock farming, which holds significant potential in terms of agricultural resources in Turkey, should prevent declines in milk and dairy product production despite high input costs. Effective management of a well-structured supply chain in livestock farms, where milk production is a primary source of livelihood, can establish a balance between costs and income.

Other challenges in dairy farming include issues related to government support, organization, roughage production, input procurement, milk price levels, and bargaining power of producers in sales. In Turkey, addressing these issues requires increased supervision in milk procurement and dairy product sales, educating milk producers on quality milk production, and maintaining a pricing mechanism based on quality (Uzmay et al., 2006:52). Therefore, making the right decisions in supplier selection for dairy farms can mitigate costs and contribute to increased production.

In conclusion, this study provides an in-depth analysis of supply chain management and supplier selection in the dairy production sector in Turkey. The literature review and field research findings indicate that dairy farms prioritize factors such as price, quality, service, and delivery time in supplier selection, alongside more specific criteria like reliability, innovation, and sustainability. Particularly, selecting the right suppliers is crucial for enhancing operational efficiency of dairy farms and ensuring their long-term sustainability.

Proposed policy and implementation steps could strengthen dairy farms' competitiveness and position in the market. Key among these steps are focusing on digitalization and technological innovations in supply chain processes, utilizing education and consultancy services to enhance efficiency, and adopting sustainable agricultural practices.

Finally, considering Turkey's agricultural potential, effective management of supply chain in the dairy production sector will enable farms to balance costs with revenues and contribute to sustainable growth in the sector.

REFERENCES

Agrawal, V., Agrawal, A. M., & Mohanty, R. P. (2017). Application of fuzzy MCDM in supplier selection of fertilizer manufacturing industry. *International Journal of Business Performance and Supply Chain Modelling*, *9*(2), 133-159.

Akbay, C., Şahin, A., Tümer, E.İ., Bilgiç, A. & Boz, İ. (2015). Türkiye'de Süt Sığırcılığında Uygulanan Desteklerin İşletme Karlığı Üzerine Olan Etkileri. [The Effects of Supports Applied in Dairy Cattle Breeding in Turkey on Business Profitability]. Tübitak Project Number:1120824, 113

Altuntaş, B., & Özdemir, H. Ö. (2021, 6-7 December). Tedarikçi Seçimini Etkileyen Kriterlerin Analitik Ağ Süreci Yöntemine Göre Belirlenmesi: Sığır Besiciliği Çiftliklerinde Bir Uygulama.[Determining the Criteria Affecting Supplier Selection According to the Analytical Network Process Method: An Application in Cattle Breeding Farms]. G. Telli and M. Civelek (Ed.) Proceedings of the International Congress on Business and Marketing'21*, İstanbul,(s.* 287-303). Publications of Doğuş University, ISBN 978-605-62095-9-8

Anggrahini, D., Baihaqi, I., & Anggani, P. C. (2018). Supplier Selection Framework for Dairy Industry in Indonesia. In 8th International Conference on Industrial Engineering and Operations Management, IEOM 2018 (pp. 2917-2926). IEOM Society.

Ataseven, Z.Y. (2023). Durum Tahmini Süt ve Süt Ürünleri. [Status Estimate Milk and Dairy Products]. *TEPGE Publication* Number:372. ISBN: 978-625-8451-92-4

Balaban, A. (2006). *Edirne İli'nde Süt Sığırcılığının Üretim, Fiyat ve Pazarlama Politikalarının Değerlendirilmesi.* Yüksek Lisans Tezi. Trakya Üniversitesi Fen Bilimleri Enstitüsü. [Evaluation of Production, Price and Marketing Policies of Dairy Cattle in Edirne Province. Master's Thesis. Trakya University Institute of Science and Technology]. Edirne.

Baltacı, A. (2018). Nitel Araştırmalarda Örnekleme Yöntemleri ve Örnek Hacmi Sorunsalı Üzerine Kavramsal Bir İnceleme. *Bitlis Eren*

Üniversitesi Sosyal Bilimler Enstitüsü Dergisi, [A Conceptual Review on Sampling Methods and Sample Size Issues in Qualitative Research. *Bitlis Eren University Social Sciences Institute Journal*].7(1), 231-274.

Bilge, E. (2018). *Kurumsal müşterilerin banka tercihinde çok kriterli karar verme yöntemleri: Aydın ilinde bir uygulama*, Aydın Adnan Menderes Üniversitesi Sosyal Bilimler Enstitüsü, İşletme Ana Bilim Dalı Yüksek Lisans Tezi, [Aydın Adnan Menderes University, Institute of Social Sciences, Department of Business Administration Master's Thesis]. Aydın.

Bircan, K. (2016). Yaş Meyve-Sebze Toptancı Pazarı İçin Lojistik Sistem Modellemesi. *Adnan Menderes Üniversitesi Sosyal Bilimler Enstitüsü Dergisi*, [Logistics System Modeling for Fresh Fruits and Vegetables Wholesale Market. *Adnan Menderes University Social Sciences Institute Journal*]. *2*(3), 1-14.

Bayramoğlu, Z. (2003). *Konya İli'nde Süt Sığırcılığı Projesi Kapsamında Yer Alan İşletmelerin Ekonomik Analizi.* Selçuk Üniversitesi Fen Bilimleri Enstitüsü Tarım Ekonomisi Anabilim Dalı, Yüksek Lisans Tezi, [Economic Analysis of Enterprises Within the Scope of Dairy Cattle Breeding Project in Konya Province. Selçuk University Institute of Science, Department of Agricultural Economics, Master's Thesis]. Konya.

Beil, D. R. (2010). Supplier selection. Wiley Encyclopedia of Operations Research and Management Science, edited by James J. Cochra. s:1 – 14. E-ISBN9780470400531 https://doi.org/10.1002/9780470400531.eorms0852

Bulut, O. D. & Paksoy, M., 2023, Süt Sığırcılığı İşletmelerinin Üst Birlik Durumuna Göre Ekonomik Analiz: Aksaray İli Örneği, *Türk Tarım ve Doğa Bilimleri Dergisi* [Economic Analysis According to the Upper Union Status of Dairy Cattle Enterprises: Aksaray Province Example, *Turkish Journal of Agriculture and Natural Sciences*]. *10*(4), 1181-1191.

Büyüközkan, G., & Vardaloğlu, Z. (2008). Yeşil Tedarik Zinciri Yönetimi. *Lojistik Dergisi*, [Green Supply Chain Management. *Journal of Logistics*]. *8*, 1-15.

Çelik, M. (2002). Batı Akdeniz Bölgesinde Süt ve Süt Ürünleri Sektörünün Stratejik Durum Analizi Ve Gelişme Olanakları. *Akdeniz Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi*, [Strategic Situation Analysis and Development Opportunities of the Milk and Dairy Products Sector in the Western Mediterranean Region. *Akdeniz University Faculty of Economics and Administrative Sciences Journal*]. *2*(4), 43-83.

Çelik, C. & Sarıözkan, S. (2017). Kırşehir İli Merkez İlçede Sığır Besiciliği Yapan İşletmelerin Ekonomik Analizi, *Harran Üniversitesi Veteriner Fakültesi Dergisi,* [Economic Analysis of Cattle Breeding Enterprises in the Central District of Kırşehir Province, *Journal of Harran University Faculty of Veterinary Medicine*]. 6 (1): 38-45

De Boer, L., Labro, E., & Morlacchi, P. (2001). A review of methods supporting supplier selection. *European journal of purchasing & supply management*, *7*(2), 75-89.

Deste, M., & Yurttaş, A. (2018). Süt işletmelerinde tedarikçi seçim kriterlerinin belirlenmesine ve değerlendirme sisteminin kurulmasına yönelik bir araştırma. *Anemon Muş Alparslan Üniversitesi Sosyal Bilimler Dergisi*, [A research on determining supplier selection criteria and establishing an evaluation system in dairy farms. Anemon Muş Alparslan University Journal of Social Sciences]. *6*(ICEESS'18), 185-194.

Ecer, F., & Küçük, O. (2008). Tedarikçi seçiminde analitik hiyerarşi yöntemi ve bir uygulama. *Atatürk Üniversitesi Sosyal Bilimler Enstitüsü Dergisi*,[Analytical hierarchy method and an application in supplier selection. *Atatürk University Social Sciences Institute Journal*]. *11*(1), 355-369.

Echefaj, K., Charkaoui, A., Cherrafi, A., Garza-Reyes, J. A., Khan, S. A. R., & Chaouni Benabdellah, A. (2023). Sustainable and resilient supplier selection in the context of circular economy: an ontology-based model. *Management of Environmental Quality: An International Journal*, *34*(5), 1461-1489.

Erkan Can M, & Boğa M. (2019). Niğde İli Sığırcılık İşletmelerinde Atık Yönetimi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi* [Waste Management in Cattle Farms in Niğde Province.

Kahramanmaraş Sütçü İmam University *Journal of Agriculture and Nature*]. 22(2):260- 269. DOI: 10.18016/ksutarimdoga.vi.469383

Eymen, U. E. (2007). Tedarik zinciri yönetimi. *Kalite Ofisi Yayınları*,[Supply chain management. *Quality Office Publications*]. *15*.

Görçün, Ö. F. (2020). Selection of Fuel Supplier for Transport Firms By Using the AHP. *Journal of Management and Economics Research*, *18*(1), 20-35.

Erkara, C. (2021). E-Tedarik Zinciri Yönetimi. Sosyal Bilimlerde E- Uzantılı Kavramlar [E-Supply Chain Management. E-Extended Concepts in Social Sciences]. Ed. C. Hatipoğlu, 1st Edition, Efe Akademi, pp.61-80, İstanbul. ISBN: 978-625-7664-35-6

Güler, D., & Saner, G. (2017). Türkiye'de Süt Sığırcılığı İşletmelerinde Tedarik Zinciri Yönetiminin Değerlendirilmesi. *Tarım Ekonomisi Dergisi, [*Evaluation of Supply Chain Management in Dairy Cattle Enterprises in Turkey. *Journal of Agricultural Economics,].* 23(2), 165-171.

Güler, D. & Saner, G. (2020). Süt Sığırcılığı İşletmelerinde Yem Tedarikçi ve Süt Alıcı Tercihlerinin Belirlenmesi: İzmir ve Manisa Örneği, *Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi, [*Determining Feed Supplier and Milk Buyer Preferences in Dairy Cattle Enterprises: Izmir and Manisa Example, Journal of Adnan Menderes University Faculty of Agriculture]. 17(2), 207-214.

Gündüz, O. & Dağdeviren, M. (2011). Bafra İlçesinde Süt Maliyetinin Belirlenmesi Ve Üretimi Etkileyen Faktörlerin Fonksiyonel Analizi. Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi,[Determination of Milk Cost and Functional Analysis of Factors Affecting Production in Bafra District. Yüzüncü Yıl University Faculty of Agriculture Journal of Agricultural Sciences]. 21(2),pp.104-111.

Gündüz, O., Kılıç, O., Emir, M. & Aydın, G. (2013). Süt ve Süt Ürünleri Tüketiminde Tüketici Tercihlerini Etkileyen Faktörler: Samsun İli Örneği, Gıda Teknolojileri Elektronik Dergisi, [Factors Affecting Consumer Preferences in Milk and Dairy Products Consumption: Samsun Province Example, Electronic Journal of Food Technologies]. 8(1), pp.36-43.

Günlü, A. (1997). Konya İli Süt Sığırcılık İşletmelerinde Karlılık Ve Verimlilik Analizleri İle İşletmelerin Üretim Ve Pazarlama Sorunları. Ankara

Üniversitesi, Sağlık Bilimleri Enstitüsü, Hayvancılık İşletme Ekonomisi Anabilim Dalı. Doktora Tezi. [Profitability and Efficiency Analyzes in Dairy Cattle Enterprises in Konya Province and Production and Marketing Problems of the Enterprises. Ankara University, Institute of Health Sciences, Department of Livestock Business Economics. Doctoral Thesis]. Konya.

Günlü, A. & Sakarya, E. (2001). Konya İli Süt Sığırcılık İşletmelerinde Karlılık ve Verimlilik Analizleri İle İşletmelerin Üretim ve Pazarlama Sorunları. *Veteriner Bilim Dergisi*, [Profitability and Efficiency Analyzes and Production and Marketing Problems of Dairy Cattle Enterprises in Konya Province. Journal of Veterinary Science]. 17(1), 97-105.

Houlihan, J.B. (1985). International Supply Chain Management, *International Journal of Physical Distribution & Materials Management*, Vol. 15 No. 1, pp. 22-38. https://doi.org/10.1108/eb014601

İçöz ,Y. (2004). Bursa İli Süt Sığırcılığı İşletmelerinin Karlılık Ve Verimlilik Analizi. Ankara Üniversitesi Sağlık Bilimleri. Doktora Tezi. [Profitability and Productivity Analysis of Dairy Cattle Enterprises in Bursa Province. Ankara University Health Sciences. Doctoral Thesis]. Ankara.

Kapar, K. (2013). Bir üretim işletmesinde analitik hiyerarşi süreci ile tedarikçi seçimi. *Dokuz Eylül Üniversitesi İktisadi İdari Bilimler Fakültesi Dergisi*, [Supplier selection by analytical hierarchy process in a manufacturing company. Journal of Dokuz Eylül University Faculty of Economics and Administrative Sciences]. *28*(1), 197-231.

Keskin, G. & Dellal, İ. (2011). Trakya Bölgesinde Süt Sığırcılığı Üretim Faaliyetinde Brüt Kar Analizi. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* [Gross Profit Analysis in Dairy Cattle Production Activity in the Thrace Region. *Kafkas University Faculty of Veterinary Medicine Journal*]. 17(2): 177-182

Murat, H. & Sakarya, E. (2012). Orta Anadolu Bölgesi Damızlık Sığır Yetiştirici Birliklerine Bağlı Süt Sığırcılığı İşletmelerinin Ekonomik Analizi. *Veteriner Hekimler Derneği Dergisi* [Economic Analysis of Dairy Cattle Enterprises Affiliated to Breeding Cattle Breeders'

Associations in the Central Anatolia Region. *Journal of the Veterinary Medical Association*]. 83(1): 5-12.

Nedeljković, M., Puška, A., & Đokić, M. (2022). Evaluation of criteria when selecting agricultural machinery suppliers. Book of Proceedings. XI International Symposium on Agricultural Sciences AgroReS, 26-28 May 2022, Trebinje, Bosnia and Herzegovina

Nizam, S., & Armağan, G. (2006). Aydin İlinde Pazara Yönelik Süt Siğirciliği İşletmelerinin Verimliliklerinin Belirlenmesi. *Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi* [Determination of Efficiency of Market-Oriented Dairy Farms in Aydin Province. Adnan Menderes University Faculty of Agriculture Journal*]. 3*(2), 53-60.

Özdemir, A. İ. (2004). Tedarik zinciri yönetiminin gelişimi, süreçleri ve yararları. *Erciyes Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi* [Development, processes and benefits of supply chain management. Erciyes University Faculty of Economics and Administrative Sciences Journal], 23.

Özüdoğru, T. (2010). Amasya Damızlık Sığır Yetiştiricler Birliğinin Yöre Çiftçilerine Ekonomik Etkilerinin Analizi. Ankara Üniversitesi Fen Bilimler Ensttüsü, Tarım Ekonomisi Anabilim Dalı, Doktora Tezi,[Analysis of the Economic Effects of Amasya Cattle Breeders Association on Local Farmers. Ankara University Institute of Science, Department of Agricultural Economics, Doctoral Thesis]. Ankara.

Strauss, A., & Corbin, J. (1998). Basics of Qualitative Research: Techniques and Procedures for Developing Grounded Theory.2nd Edition. Sage Publications, Inc. E-book ISBN: 9780585383323

Soyak, A., Soysal, M.İ. & Gürcan, E.K. (2007). Tekirdağ İli Süt Sığırcılığı İşletmelerinin Yapısal Özellikleri Ve Bu İşletmelerdeki Siyah Alaca Süt Sığırlarının Çeşitli Morfolojik Özellikleri Üzerine Bir Araştırma. *Tekirdağ Ziraat Fakültesi Dergisi* [A Research on the Structural Characteristics of Dairy Cattle Farms in Tekirdağ Province and Various Morphological Characteristics of Holstein Friesian Dairy Cattle in These Farms. Journal of Tekirdağ Faculty of Agriculture]. 4(3): 297-305.

Şahin, K., Gül, A., Koç, B. & Dağıstan, E., 2001. Adana İlinde Entansif Süt Sığırcılığı Üretim Ekonomisi. *Yüzüncü Yıl Üniversitesi Ziraat*

Fakültesi Tarım Bilimleri Dergisi [Intensive Dairy Cattle Production Economy in Dana Province. Yüzüncü Yıl University Faculty of Agriculture Journal of Agricultural Sciences]. 11(2): 19-28.

Şahin, K. & Karadağ Gürsoy, A. (2016). Iğdır İli Süt Sığırcılığı İşletmelerinin Sosyo Ekonomik Yapısı, *Nevşehir Bilim ve Teknoloji Dergisi Targid* Özel Sayı [Socio-Economic Structure of Dairy Cattle Enterprises in Iğdır Province, Nevşehir Science and Technology Magazine Targid Special Issue]. 118-129 2016

Talim, M., Saner, G., Karahan,Ö. & Engindeniz, S. (2000). Türk-Anafi Projesi Kapsamındaki Süt Sığırcılığı İşletmelerinde Prodüktivite Ve Rantabilite Üzerine Bir Araştırma. Ege Üniversitesi Rektörlüğü Araştırma Fonu, Proje No: 1994/Zrf-013. [A Research on Productivity and Profitability in Dairy Cattle Enterprises within the Scope of Türk-Anafi Project. Ege University Rectorate Research Fund, Project No: 1994/Zrf-013]. Bornova/ İzmir, ISBN:975-96867-3-2.

Tandoğan, M.. (2006). *Afyonkarahisar Süt Sığırcılık İşletmelerinde Karlılık Analizi İle İşletmelerde Karşılaşılan Üretim ve Pazarlama Sorunları.* Afyon Kocatepe Üniversitesi Sağlık Bilimleri Enstitüsü. Yüksek Lisans Tezi [Profitability Analysis in Afyonkarahisar Dairy Cattle Enterprises and Production and Marketing Problems Encountered in the Enterprises. Afyon Kocatepe University Institute of Health Sciences. Master's Thesis]. Afyon.

Tamulienė, V., Raupelienė, A., & Kazlauskienė, E. (2017). Farmers' preferences selecting agricultural consulting services. *Montenegrin journal of economics. Podgorica: Economic laboratory on transition research.* Vol. 13(4).

Turan, E., Ağlargöz, O., Çınar, E., Yılmaz, H., & Yapıcıoğlu, H., (2012). E-İş Süreçleri, Tedarik Zinciri Yönetimi. Anadolu Üniversitesi Yayını No:2493 [E-Business Processes, Supply Chain Management. Anadolu University Publication No: 2493]. Eskişehir.

TÜİK (5 Mayıs 2023). Çiğ Süt Üretim İstatistikleri, 2022. Türkiye İstatistik Kurumu Haber Bülteni Sayı:49699 [Raw Milk Production Statistics, 2022. Turkish Statistical Institute Newsletter Number: 49699]. www.tuik.gov.tr Date of access: 18 May 2024

Türkyılmaz, M.K. & Aral, S., 2002. Aydın İli Süt Sığırcılık İşletmelerinde Kaynak Kullanımında Etkinlik Derecesi ile Örgütlenme ve Pazarlama Sorunları [in Turkish]. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 8(1): 41-48.

Ünal, N.R. & Besler, H.T. (2012). Beslenmede Sütün Önemi. Sağlık Bakanlığı Yayınları, 2. Basım, Yayın No:727 [The Importance of Milk in Nutrition. Ministry of Health Publications, 2nd Edition, Publication No: 727]. ISBN: 978-975-590-243-2, Ankara.

Uyanık, S. (2000). Burdur İli Süt Sığırcılık İşletmelerinde Karlılık Ve Verimlilik Analizleri [Profitability and Efficiency Analysis of Dairy Farming Enterprises in Burdur Province]. Ankara Üniversitesi Sağlık Bilimleri Enstitüsü. Doktora Tezi [Ankara University Institute of Health Sciences. Doctoral Thesis]. Ankara.

Uzmay, A., Koyubende, N. & Konca, Y. (2006). İzmir İlinde Süt Ve Süt Ürünleri İşleyen ve Pazarlayan İşletmelerin Bazı Özellikleri Üzerine Bir Araştırma, [A Study on Some Characteristics of Dairy Processing and Marketing Enterprises in Izmir Province]. *Ege Üniversitesi. Ziraat Fakültesi Dergisi*, [*Ege University Faculty of Agriculture Journal*] 43 (3):43-53 Issn 1018-8851

Van der Lee, J., Bebe, B. O., & Oosting, S. (2016). *Sustainable intensification pathways for dairy farming in Kenya: A case study for PROIntensAfrica WP2, Deliverable 2.3* (No. 997). Wageningen Livestock Research.

Van Thanh, N., Lan, N. T. K., Husain, S. T., Hai, N. H., & Tinh, N. V. (2022). Optimization agricultural supply chain: a case study of fertilizer supplier selection. *Cmc-Computers Materials & Continua*, *72*, 4057-4068.

Yıldırım, İ. & Şahin, A., 2003. Van İli Merkez İlçede Süt Sığırcılığı Yapan İşletmelerin Ekonomik Analizi. [Economic Analysis of Dairy Farms Operating in the Central District of Van Province]. *Van Ticaret Borsası Yayınları* [Van Chamber of Commerce Publications]. Sayı:1.

Yılmaz, H., Ayasan, T., Sağlam, C. & Gül, M. (2019). Socio-Economic Characteristics of Dairy Farms and Use Level of Feedstuff in The Eastern Mediterranean Region, *Turkish Journal of Agriculture - Food*

Science and Technology, 8(1): 89-94, https://doi.org/10.24925/turjaf.v8i1.89-94.2749

CHAPTER 16

STUDY ON THE BIOLOGICAL ACTIVITY OF *Salix babylonica* L. PLANT: ANTIFUNGAL AND ALLELOPATHIC EFFECT

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1. INTRODUCTION

The family *Salicaceae*, which includes weeping willows, consists of deciduous trees or shrubs. This family is distributed worldwide, except in Australia, and is represented by approximately 550 species (Arıhan, 2003). Within this family, the genus Salix (willows) comprises around 520 species found in the cold and temperate regions of the Northern Hemisphere (Skvortsov and Edmondson, 1982; Anonymous, 2024). In Turkey, 27 taxa belonging to the genus Salix have natural distribution (Terzioğlu et al., 2014).

Salix babylonica, commonly known as the weeping willow, belongs to the genus Salix within the Salicaceae family and is one of the best-known willow species, distributed in parts of Asia, Europe, and America. It is considered an important tree for the study of its phytochemical properties due to its use in ornamental and medicinal purposes (Abdel Wahab et al., 2018). *Salix babylonica* contains compounds such as benzyl ester of gentisic acid 20-O-acetyl -d-glucoside, trichocarpin, salicin, kaempferol 7-O-glucoside, apigenin 7 O-galactoside, luteolin 40-O-glucoside, ester of terephthalic acid, tritetracontane, octadecenoic acid-1,2,3-propanetriyl ester, hexadecenoic acid-methyl ester, and 1,3-dioxane-4-(hexadecyloxy)-2-pentadecyl. These compounds exhibit antiseptic, analgesic, anticancer, antipyretic, antimalarial, antioxidant, antifungal, and antibacterial activities (Salem et al., 2011; Wahab et al., 2018; González-Alamilla et al., 2019).

The water extract of *S. babylonica* has allelopathic effects on *Lactuca sativa* L. (lettuce), *Lavandula stoechas* L. (French lavender), and *Echium plantagineum* L. (purple viper's bugloss) plants (Chaves Lobón et al., 2023). It has been reported that the leaf extracts of *S. babylonica* have allelopathic effects on Chlorella pyrenoidosa, depending on the concentration (Li Qing-hua et al., 2009). Leachate from plant residues of *Salix caprea* in the genus Salix inhibited the germination, shoot, and root growth of three plant species (*Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl, *Plantago lanceolata* L., and *Lotus corniculatus* L.) (Mudrák and Frouz, 2012). Ahmed et al. (2016) reported that the methanol extract of *Salix nigra* inhibited wheat root and shoot growth compared to the control.

Medicinal plants are important in various fields such as health and cosmetics. This study aims to reveal the allelopathic and antifungal potential of the medicinally significant *Salix babylonica*.

2. MATERIALS AND METHODS

2.1. Collection and Drying of Plant Material

Salix babylonica plant material was collected from the natural area in Kırşehir during the 2022-2023 vegetation period. The collected plant material was dried in the shade in the laboratory and ground using an electric grinder, then stored in paper bags in the shade.

2.2. Preparation of Plant Extract

100 g of ground plant material was weighed into a 1-liter Erlenmeyer flask, and 600 ml of methanol was added. The mixture was shaken in an orbital shaker at 120 rpm at room temperature for 72 hours. After this period, the extract was filtered, and the methanol was removed using an evaporator. The remaining solid was dissolved in dimethyl sulfoxide (DMSO)-pure water to prepare different concentrations (Yılar and Bayar, 2023).

2.3. Effect of Plant Extracts on Seed Germination and Seedling Growth of Test Plants

The experiments to investigate the effect of the plant extract on seed germination and seedling growth were conducted in 9 cm diameter Petri dishes. Twenty-five seeds of the test plant were evenly distributed on drying paper placed in two layers in Petri dishes. Different concentrations 0 (control), 1, 2, and 4 mg mL^{-1} of the prepared plant extract and control (DMSO-pure water) were used, and 5 ml was added to the Petri dishes to moisten them. The Petri dishes were incubated at an average of 24°C for 1-3 weeks. At the end of this period, the germination rate of the seeds and the lengths of the roots and shoots were determined (Önen, 2003). The seed germination inhibition rate (%) of Salix babylonica extract was calculated using the following formula

$$I:100x(dc-dt)/dc$$

Where: I = Seed germination inhibition(%) dc = Germination rate (%) in control dt = Germination rate (%) in treatment

Additionally, a study was conducted to determine the effect of plant extracts on seedling growth after germination. Three plants with 2 mm root growth, which had previously germinated in Petri dishes, were placed between two filter papers with 5 ml of extract applied. At the end of 7 days, the lengths and dry weights of the plants were determined. The experiments were conducted with 4 replications and repeated twice.

2.4. Obtaining Fungal Cultures

Fusarium oxysporum f. sp. lycopersici and *F. oxysporum* f. sp. *melonis* fungi were obtained from stock cultures in Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, Phytoclinic laboratories. In the experiments, young fungal cultures developed from these stock cultures for 7 days at 25±2°C in 90 mm Petri dishes containing 20 ml of potato dextrose agar (PDA) were used.

2.5. Antifungal Activity of Plant Extract in In Vitro Conditions

The extract obtained from *Salix babylonica* was dissolved in a mixture of 1% DMSO to obtain a stock solution. From the original solutions obtained, final concentrations (0 (control), 1, 2, and 4 mg mL^{-1}) were added to PDA media cooled to 45-50°C. As a control, fungi were only plated on Petri dishes containing PDA. Additionally, a fungicide with the active ingredient Thiram was used as a positive control. These PDA media at different doses were poured into 60 mm diameter Petri dishes at 10 ml each. 5 mm diameter mycelium discs taken from 7-10 day-old plant pathogen cultures were inoculated into Petri dishes containing PDA media with extract. The fungal cultures were incubated at 25±1°C for 7 days in a growth chamber after inoculation. This study was repeated twice with 3 replications. The diameters of the developed mycelium in Petri dishes were measured with a digital caliper. The mycelium growth inhibition percentage of Salix babylonica extract was calculated using the following formula:

$$I:100x(dc-dt)/dc$$

Where: I = Mycelium growth inhibition(%) dc = Mycelium growth in control dt = Mycelium growth in treatment

2.6. Data Analysis

The significance levels of the differences between treatments in the experiments were determined by variance analysis (ANOVA), and the means were compared using the DUNCAN test. Statistical analyses were performed using the SPSS 15 computer program.

3. RESULTS AND DISCUSSION

3.1. Allelopathic Effect of *Salix babylonica* Methanol Extract on *Panicum miliaceum*

The methanol extract of *Salix babylonica* had a generally negative effect on seed germination, root, and shoot development of *Panicum miliaceum*. The adverse effect varied depending on the increasing dose. The inhibitory effect of the plant methanol extract on *P. miliaceum* seed germination, root, and shoot development was found to be statistically significant ($p<0.05$) (Table 1, Table 2, Figure 1, Figure 2).

Table 1. Effect of *Salix babylonica* Methanol Extract on Seed Germination, Shoot, and Seedling Growth of *Panicum miliaceum* (Broomcorn Millet)

Doses (mg mL^{-}	Germination Rate (%)	Root Length (cm)	Shoot Length (cm)
Control	$93{,}00^{a*}\pm1{,}91$	$7{,}40^{a}\pm0{,}34$	$2{,}93^{a}\pm0{,}07$
1	$86{,}00^{a}\pm3{,}46$	$4{,}91^{b}\pm0{,}20$	$2{,}97^{a}\pm0{,}12$
2	$70{,}00^{b}\pm3{,}46$	$2{,}90^{c}\pm0{,}10$	$2{,}20^{b}\pm0{,}04$
4	$55{,}00^{c}\pm2{,}51$	$1{,}30^{d}\pm0{,}04$	$0{,}75^{c}\pm0{,}05$

[*] Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05)

The plant extract inhibited *P. miliaceum* seed germination by 40.86% at the highest dose (4 mg/ml) compared to the control. Additionally, it inhibited root growth by 82.43% and shoot growth by 74.40% compared to the control (Figure 1).

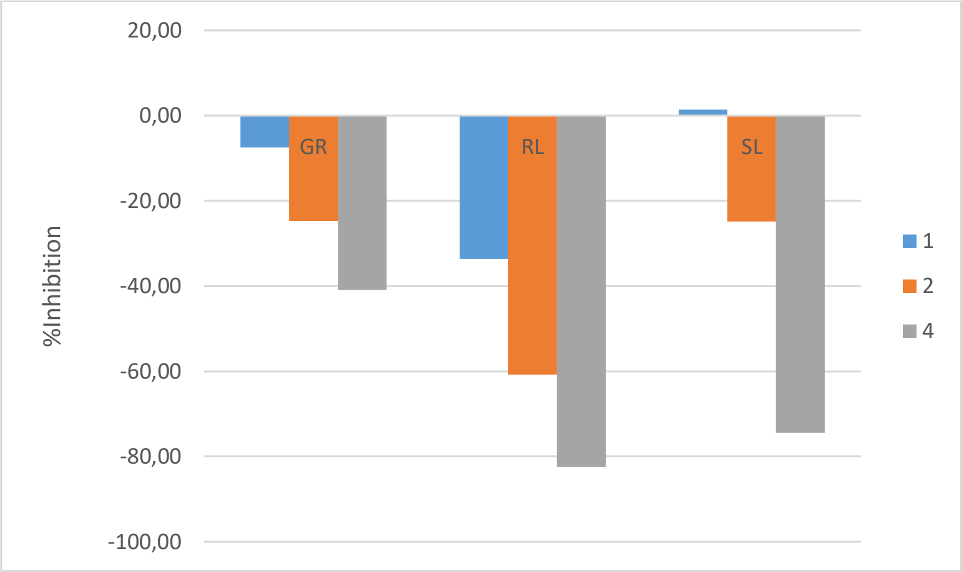

Figure 1. *Panicum miliaceum* GR, RL and SL % inhibition rates of *Salix babylonica* methanol extract(1, 2, 4 mg/mL)

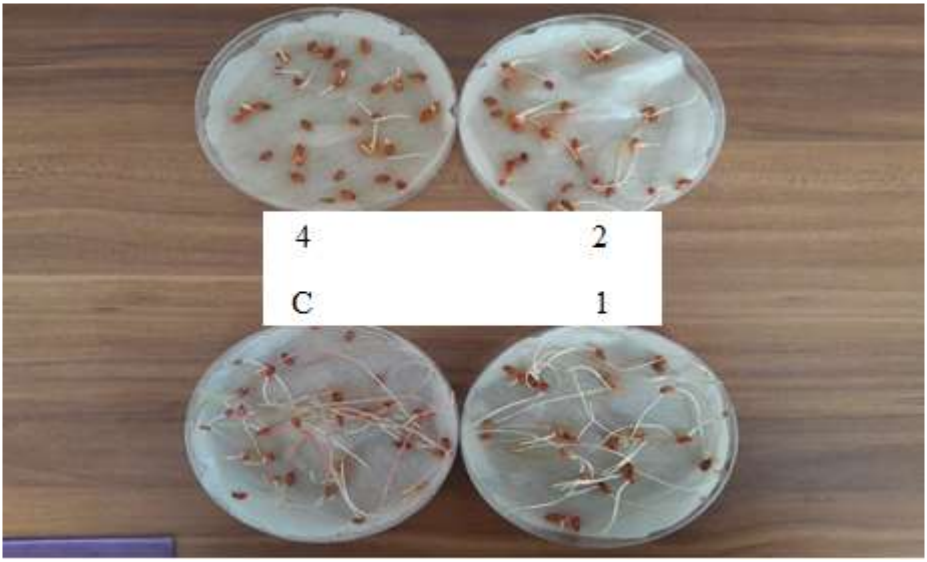

Figure 2. Effect of *Salix babylonica* methanol extract (1, 2, 4 mg/mL) on *Panicum miliaceum* germination and seedling emergence.

In the experiment, the application of the extract to seedlings that had germinated and exhibited 2 mm root growth yielded results similar to those of the germination experiment. Accordingly, the 1 mg/mL dose of *S. babylonica* extract inhibited *P. miliaceum* seedling root growth by 33.31%, the 2 mg/mL dose by 48.31%, and the 4 mg/mL dose by 74.92% compared to the control. Unlike root growth, the 1 and 2 mg/mL doses of the extract exhibited a stimulatory effect on the shoot growth of *P. miliaceum*. However, the 400 mg/mL dose of the extract inhibited *P. miliaceum* shoot growth by 43.50% compared to the control (Table 2, Figure 3, Figure 4).

Table 2. Effect of Different Concentrations of *Salix babylonica* Methanol Extract on Root and Shoot Development of *Panicum miliaceum*

Doses (mg mL^{-1})	**Root**	**Shoot**
Control	13,00^{a*}±0,41	5,77^{a}±0,73
1	8,67^{b}±0,61	6,15^{ab}±0,46
2	6,72^{c}±0,42	5,91^{ab}±1,22
4	3,26^{d}±0,51	3,26^{b}±0,51

[*] Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05)

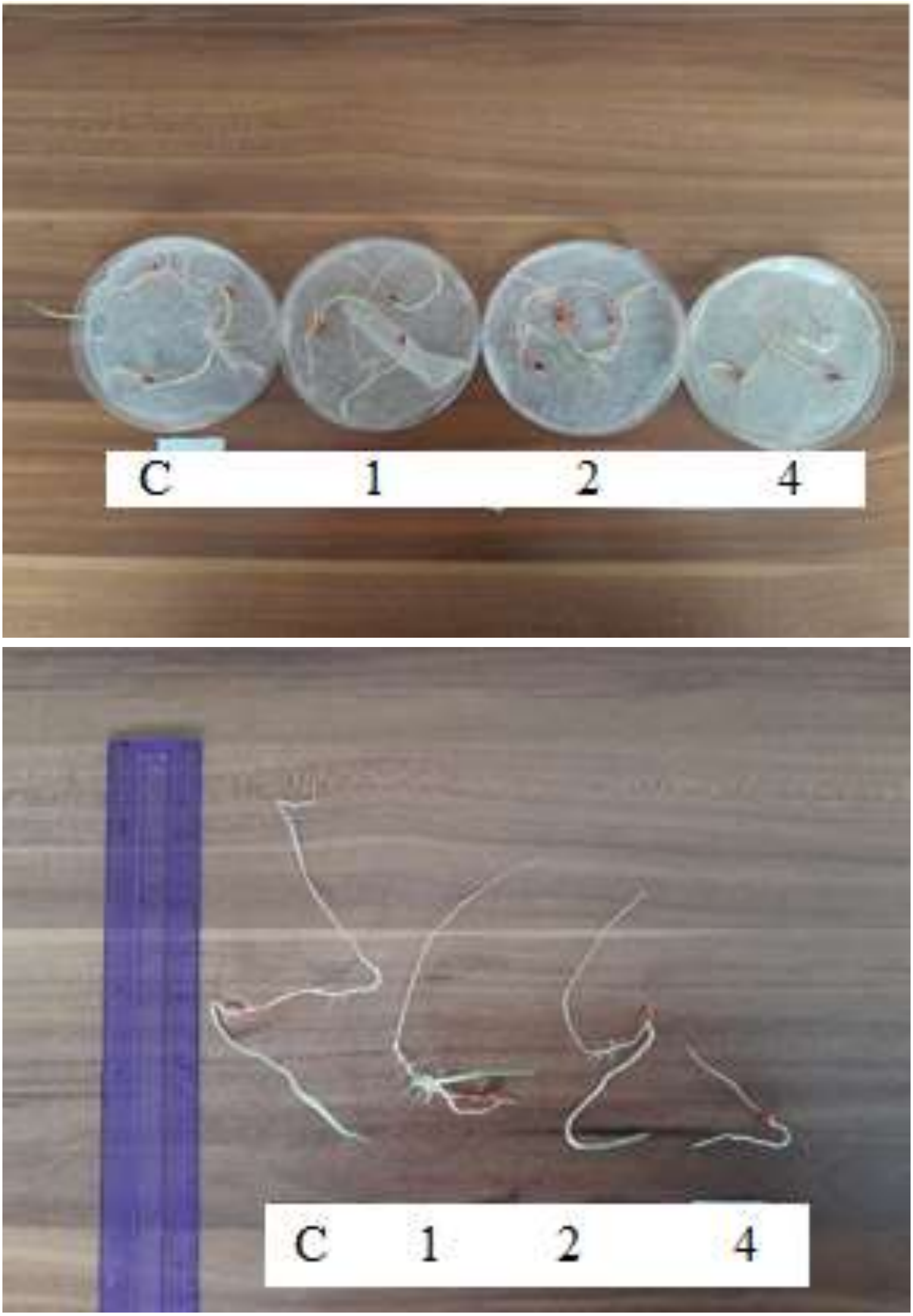

Figure 3. Effect of *Salix babylonica* methanol extract on *Panicum miliaceum* seedling development

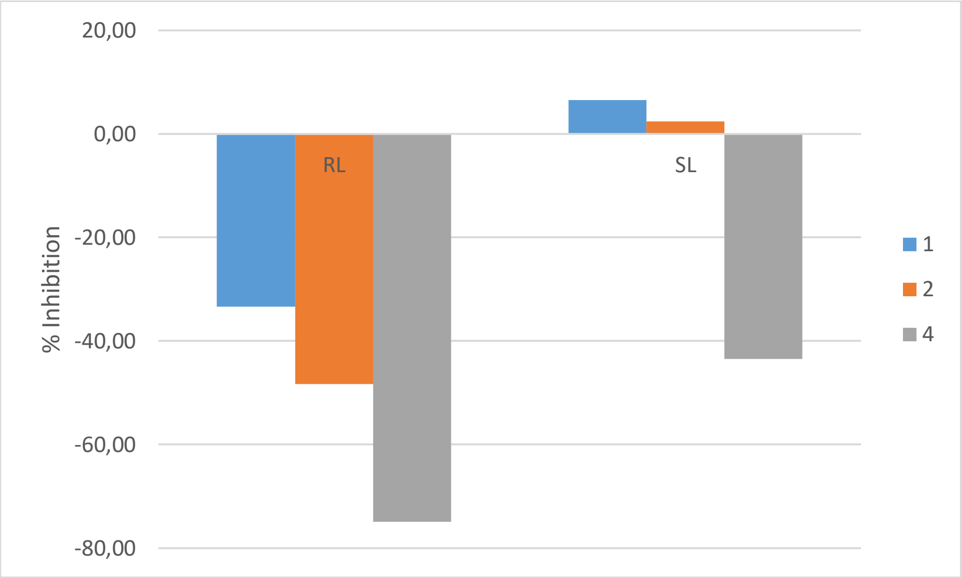

Figure 4. % inhibition rate of *Panicum miliaceum* seedling growth of *Salix babylonica* methanol extract (RL: root length; SL: Shoot length)

Willows (Salix spp.), members of the Salicaceae family, are deciduous trees or shrubs. The use of the willow tree as a significant medicinal plant dates back approximately 6000 years. For many years, willow bark and leaves were used by ancient civilizations to treat various ailments, without knowledge of their active components. The bark and leaves of the plant possess analgesic, antipyretic, and anti-inflammatory effects (Mutlu-Durak & Yıldız Kutman, 2021). The active component of the plant is the phenolic glycoside salicin, which has various biological activities (Zeid, 2006).

Studies conducted with Salix species have determined that these species exhibit allelopathic effects. The water extract of Salix nigra inhibited wheat root and shoot growth, as well as fresh and dry weight, compared to the control, in both petri dish and field applications at a significance level of $P < 0.05$ (Ahmed et al., 2016). *Fallopia japonica* plants irrigated with water extracts of *Salix fragilis* were observed for 196 days. While plant height and leaf number were not affected by the extract, roots showed a reduction of up to 32% (Koce, 2016).

It has been reported that the root mass of *Picea abies* seedlings treated with extracts of *Salix caprea* decreased (Schütt & Blaschke, 1980).

Additionally, when seeds were directly treated with leaf plant residues of *S. caprea*, a decrease in seed germination of *Arrhenaterum elatius*, *Lotus corniculatus*, and *Plantago lanceolata* was observed (Mudrak & Frouz, 2012). The water extract of *Salix babylonica* leaves exhibited various levels of allelopathic effects on the seed germination and root elongation of *Lactuca sativa*, *Lavandula stoechas,* and *Echium plantagineum* (Chaves Lobón et al., 2023). Moreover, *S. babylonica* has been found to stimulate the growth and root development of maize (*Zea mays*) (Mutlu-Durak & Kutman, 2021).

Antifungal activity: The methanol extract obtained from the leaves of *Salix babylonica* was evaluated for its effect on the mycelial growth of plant pathogens. The study results showed that the extract did not completely inhibit the mycelial growth of the tested plant pathogens. Statistically significant differences at the $P \leq 0.05$ level were found among the extract doses. The methanol extract of the plant had the most significant effect on *F. oxysporum* f. sp. *melonis*, with an inhibition rate of 34.62% compared to the negative control. However, the plant extract showed no effect on *Fusarium oxysporum* f. sp. *lycopersici* (Table 3).

Tablo 3: Effect of *Salix babylonica* Methanol Extract on Plant Pathogenic Fungi (%)

Doses (mg mL^{-1})	**Fusarium oxysporum f. sp. lycopersici**	**F. oxysporm fs p. melonis**
C+	100^{a}±0.00	100^{a}±0.00
N-	0.00^{b}±0.00	0.00^{d}±0.00
1	0.00^{b}±0.00	0.00^{d}±0.00
2	0.00^{b}±0.00	16.87^{c}±9,1
4	0.00^{b}±0.00	34,62^{b}± 2.03

*****Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05), C+ ; Positive control, N-; Negativi control

Studies on the effects of *Salix babylonica* on plant pathogens are limited. However, there are several studies involving other Salix species. In one study, the 20% ethanol extract of *Salix babylonica* was reported to inhibit the mycelial growth of the plant pathogen *Fusarium* oxysporum by 29.22% (Sati et al., 2013). Another study investigated the effect of aqueous extracts of neem (*A. indica*) and willow (*Salix babylonica*) on *Fusarium* wilt disease in tomato seedlings. Treatment of tomato plants with neem aqueous extract reduced the disease incidence to 25.5% six weeks post-infection (Farag Hanaa et al., 2011).

A study with methanol extracts of *Salix alba* bark found the extract ineffective against *A. terreus* and *R. stolonifer*. However, it exhibited activity against *A. ornatus* at 25 mg/mL, 50 mg/mL, and 75 mg/mL, with inhibition zones of 12.5±0.500, 10.333±1.528, and 12.667±2.082 mm, respectively (Javed et al., 2021). The disease control observed with willow aqueous extracts could be due to either direct antimicrobial activities or the presence of biologically active compounds that induce host plant defense responses, reducing *Fusarium* wilt development (Amadioha, 2000; Schneider and Ulrich, 1994).

Our study demonstrated that the methanol extract of *Salix babylonica* exhibited significant activity against *F. oxysporum* f. sp. *melonis*. Specifically, the methanol extract inhibited the mycelial growth of this pathogen by 34.62% at the highest concentration tested.

CONCLUSION

In conclusion, the methanol extract of *Salix babylonica* exhibited antifungal activity against two major plant pathogenic fungi (*F. oxysporum* f. sp. *melonis*). Additionally, the plant extract significantly affected seed germination, root, and shoot development of *Panicum miliaceum*. The data obtained in this study provide a basis for further research. Future studies should explore the effectiveness of the plant extract on various plant pathogens and weeds through both petri dish and field trials to support these findings.

REFERENCES

Amadioha, A.C., 2000. Controlling rice blast in vitro and in vivo with extracts of *Azadirachta indica*. *Crop Protection*, 19: 287-290

Abdel Wahab, G., Sallam, A., Elgaml, A., Lahloub, M.F., Afifi, M.S., 2018. Antioxidant and antimicrobial activities of *Salix babylonica* L. extracts. W. J. *Pharmaceut. Sci* 6 (4): 1–6.

Ahmed, W., Ahmad, M., Khan, R.A., 2016. Potential allelopathic inhibitory effect of Salix nigra on growth of *Triticum aestivum*. International Journal of Biosciences, 8(3):29-34.

Anonim,2024.3.Salix Linnaeus, Sp.Pl.2:1015.1753. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=129059(Erşimtarihi:20.04.2024)

Chaves Lobón, N., González Félix, M., Alías Gallego, J.C., 2023. Comparison of the Allelopathic Potential of Non-Native and Native Species of Mediterranean Ecosystems. *Plants*, 12(4):972.

González-Alamilla, E.N., Gonzalez-Cortazar, M., Valladares-Carranza, B., Rivas-Jacobo, M.A., Herrera-Corredor, C.A., Ojeda-Ramírez, D., Zaragoza-Bastida, A., Rivero-Perez, N., 2019. Chemical Constituents of *Salix babylonica* L. and Their Antibacterial Activity Against Gram-Positive and Gram-Negative Animal Bacteria. Molecules,18;24(16):2992.

Farag Hanaa, R.M., Abdou, Z.A., Salama, D.A., Ibrahim, M.A.R., Sror, H.A.M., (2011). Effect of neem and willow aqueous extracts on fusarium wilt disease in tomato seedlings: Induction of antioxidant defensive enzymes. *Annals of Agricultural Sciences,* 56:1-7

Javed, B., Farooq, F., Ibrahim, M., Abbas, H.A.B., Jawwad, H., Zehra, S.S., Ahmad, H.M., Sarwer, A., Malik, K., Nawaz, K., 2021. Antibacterial and antifungal activity of methanolic extracts of *Salix alba* L. against various disease causing pathogens. *Braz J Biol.*83:e243332

Koce, J.D., 2016. The effects of leaf extracts of crack willow (*Salix fragilis*) on the growth of Japanese knotweed (*Fallopia japonica*). *Acta Biologica Slovenica*, 59 (1):13-21.

Li , Q.H. , Guo , P.Y., Tian, M.Y. and Jiang, Z.Y., 2009. Allelopathy of *Salix babylonica* leaf extracts on *Chlorella pyrenoidosa* .*Chinese Journal of Applied Ecology* , 28 (5): 884 – 888

Mudrák, O., Frouz, J., 2012. Allelopathic effect of *Salix caprea* litter on late successional plants at different substrates of post-mining sites: pot experiment studies..*Botany,* 90(4):311-318.

Mutlu-Durak, H.,Yildiz Kutman, B., 2021. Seed treatment with biostimulants extracted from WeepingWillow (*Salix babylonica*) enhances early maize growth. *Plants*, 10, 1449.

Önen, H., 2003. Bioherbicidal effects of some plant essential oils, Turkish Herbology Journal, 6 (1): 39-47.

Schneider, S., Ultrich, W.R., 1994. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. *Physiological and Molecular Plant Pathology*, 45: 291-304

Sati, S.C., Singh, H., Badoni, P.P., and Sati, M..D., 2013. Screening of Fungicidal Activity of Salix and Triumfetta Species of Garhwal Himalaya. American Journal of Phytomedicine and Clinical Therapeutics, 1(5):486-489.

Salem, A.Z.M., Olivares, M., Lopez, S., Gonzalez-Ronquillo, M., Camacho, L.M., Cerrillo, S.M.A., Mejia, H.P., Rojo, R., 2011. Effect of natural extracts of *Salix babylonica* and *Leucaena leucocephala* on nutrient digestibility and growth performance of lambs. Anim. *Feed Sci. Tech*., 170:27-34.

Schütt, P., Blaschke, H. 1980. Seasonal changes in the allelo- pathic effect of *Salix caprea* foliage. *Flora,* 169(4):316-328.

Skvortsov, A.K., Edmondson, J.R. 1982. Salix. Pp. 694-716 in: Davis, RH. (ed.), Flora of Turkey and the East Aegean Islands, vol. 7. Edinburgh University Press, Edinburgh.

Terzioğlu, S., Serdar, B., Karaköse, M., Coşkunçelebi, K., Gültepe, M., 2014. New data on *Salix anatolica* (Salicaceae) endemic to Turkey. Phytotaxa 167 (1): 111–118.

Yılar, M., Bayar, Y., 2023. Lc-Ms/Ms analysıs of phenolic compounds, antifungal and allelopathic potential of *Salvia ceratophylla* L. collected from Türkiye . *Environmental Engineering and Management Journal*, 22(11):1775-1782.

Zeid, A.A., 2006. Phenolics, volatiles and biological activities of *Salix babylonica* L. leaves and stem bark. *Planta Med*, 72, P-165.

CHAPTER 17

BREEDING POLYPLOID WATERMELON

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BREEDING POLYPLOID WATERMELON

Classical plant breeding is based on planned hybridization of closely related species. In classical breeding, there is phenotypic selection of superior genotypes among the offspring obtained because of crossbreeding and showing development. In classical breeding, it may take several generations to evaluate and select useful genotypes and can take a long time. For this reason, there may be situations where classical breeding may be limited due to increasing food demands (Tester & Langridge, 2010). Classical breeding faces some limitations both because of the genotype*environment interaction and because phenotypic selection is expensive and time-consuming. In this context, the variation that can be created by intraspecific or interspecific hybridization between genotypes with the same ploidy level may be limited.

Polyploidy breeding was developed as an alternative to these problems encountered in classical plant breeding and played an important role in the evolutionary history of plants (Doyle et al., 2008; Otto & Whitton, 2003). Almost all angiosperms (angiosperms) have undergone at least one full round of genome doubling during their evolution (Adams & Wendel, 2005; Wendel, 2000). Polyploids are classified as autopolyploids and allopolyploids. Autopolyploids arise from the doubling of a diploid genome, while allopolyploids arise from the combination of two or more different sets of genomes (Chen, 2007; Doyle & Egan, 2010). Many plants are polyploid, including wheat (allohexaploid), cotton (allotetraploid), rapeseed (allotetraploid), sweet potato (autotetraploid), potato (autotetraploid). Additionally, polyploid varieties are common in plants such as banana (triploid), grape (tetraploid), kiwi and date (hexaploid), strawberry (octaploid). Phenotypic variations caused by polyploidization specifically increase biomass and stress tolerance, and as a result, agricultural productivity and efficiency also increase. The morphology and physiology of newly formed offspring are significantly affected by polyploidy. Polyploids tend to have larger cells than diploids, which results in larger leaves, flowers, and seeds (Abel & Becker, 2007; Li et al., 2012). Physiological traits such as plant height, growth rate, flowering time and reproduction can also be modified by polyploidization (Cohen & Tel-Zur, 2012; Yao et al., 2011). Previous studies have shown that tetraploidization can significantly increase stress tolerance (Chao et al., 2013; del Pozo & Ramirez-Parra, 2014).

Polyploidy is a notable trait in the development of high-yielding crop plants, and approximately half of plant species are polyploid (Adams & Wendel, 2005). In artificially obtained autopolyploids, after genome doubling, they exhibit new characters such as high DNA content and high secondary metabolite activity, large tissues and organs, increased efficiency, higher chlorophyll, lycopene, fructose and glucose content and higher tolerance. For all these reasons, they are more tolerant to both abiotic and biotic stress conditions than diploid plants, and these are all clear advantages of polyploidy breeding (Doyle & Coate, 2018; Jaskani, Kwon, & Kim, 2005; King et al., 2010; Sattler et al., 2016; Soltis et al., 2016; Zhang et al., 2019; Zhu et al., 2018).

Such beneficial phenotypic variation can also be achieved by artificially inducing the formation of polyploids. Polyploidy relates to the number of copies of the entire set of chromosomes in all cells of an individual. Chromosome sets are species specific. Plants differ in terms of ploidy level according to the number of genomes in the cell. If the cell contains chromosomes within the framework of two genomes, it is called diploid, and if it carries three or more genomes, the cell is called polyploid. Gametes can carry a single genome (haploid; monoploid), or they can carry two or more genomes (diploid, polyploid). In their natural state, plants exist in a diploid or polyploid state. Polyploid organisms containing an unequal number of homologous chromosome sets cannot continue their generation; therefore, species that continue their generation through sexual reproduction are generally triploid, pentaploid, etc. They reported that this situation was not encountered (Klug et al., 2011).

Polyploidy is beneficial in several ways. While many polyploid plants show higher vigor (survival) than their diploid ancestors, they may show a high rate of heterozygosity, a feature similar to the heterosis effect (especially alloppolyploids). Therefore, polyploids may show new and useful phenotypic variation. Such beneficial phenotypic variation can also be achieved by artificially inducing the formation of polyploids. In addition, polyploids can be useful in overcoming hybridization barriers. For example, although hybridization cannot be done at the diploid level, it should be investigated whether this is possible at the tetraploid level. Tetraploid plants are necessary as an intermediate form in obtaining triploid plants. In addition, in hybridizations between distantly related species, the sterility that occurs in F1 can be overcome at the polyploid level(Hayward et al., 1993; Rejeb & Benbadis, 1989). Many existing polyploid species, such as Brassica, emerged in this way (Briggs, 1978). In addition, it is also known that diploid plants with two genomes previously reached the polyploidy level, but over time the chromosomes began to act as diploids (Bowers et al., 2003).

Polyploidy comes in two forms; in the case of autopolyploidy, each additional set of chromosomes is identical to the ancestral species. Allopolyploidy occurs when two very close species interbreed (Klug et al., 2011). Autopolyploid plants can arise spontaneously in nature. In addition, their formation can be promoted in vivo and in vitro. These plants occur in two main ways in nature. In the first of these, the chromosome number of somatic cells doubles spontaneously. Here, cells formed in the meristem with folded chromosome numbers and cells with no change in chromosome number can develop together. In this way, chimeric tissues and organs may emerge, or the number of chromosomes may spontaneously double during injury and callus formation and regeneration in the plant, and the tissues and organs that develop from this may continue their lives as polyploid. As a result of the development of flowers and seeds from organs with folded (doubled) number of chromosomes and their use in production or the role of polyploid tissues/organs in vegetative propagation, polyploid plants can continue their lives as cultivated plants in a differentiated manner at the ploidy level. A second way for autopolyploidy to occur spontaneously in nature is gametes whose chromosome number is not reduced. The zygote they will form and the seed and plant that develop subsequently will be completely polyploid (Harlan, 1995). It is important to diagnose spontaneous or induced polyploids. For this purpose, cytology studies are basically carried out and the results obtained are associated with the morphology of the plant. The most basic study is cytology, and definitive results can be obtained by determining the chromosome number (Rejeb & Benbadis, 1989). Once this is done, some other cytological and morphological characteristics of the plant are determined and the results are related to the chromosome number. The changes seen in polyploid plants are as follows:

1. Growth in cell size and therefore tissues and organs (gigantism): Growth in flowers and leaves, larger but fewer seeds,

2. Differentiation in the shape and structure of the organs: Thickening, wrinkled appearance and increased hairiness in the leaves,
3. Change in plant physiology: differentiation in plant-water relations and photosynthesis,
4. Differentiation in the phenology of the plant: flowering occurring later and lasting longer, etc.
5. Increase in the number of chloroplasts in guard cells in stomata: Change in stoma size and number of stomata per unit area,
6. Gamete viability and therefore fertility may decrease.

The use of polyploid rootstocks or scions may change the concentration and ratio of phytohormones (ABA, IAA, BRs, auxin, ethylene, cytokinin, etc.) and will affect the phenotype of the plant (Zhao et al., 2018).. The effects of polyploid rootstocks have also been studied in other species. In the "Kinnow" mandarin variety, which was grafted on three different rootstocks and treated with chromium toxicity, it was determined that the plants grafted on tetraploid rootstocks were more tolerant to chromium toxicity than the plants grafted on diploid rootstocks (Balal et al., 2017). In citrus, a study reported that tetraploid rootstocks showed higher salt tolerance than diploid ones (Ruiz et al., 2016; Saleh et al., 2008). In the study using diploid, triploid and tetraploid watermelon rootstocks, they explained that after grafting, tetraploid watermelon had higher hormone content and higher interaction between cytokinin and auxin than diploid and triploid (Kaseb et al., 2020). Rootstocks play an important role in controlling scion growth and development through modulation of hormone signaling pathways (Adams & Wendel, 2005; Corso et al., 2016; Gregory et al., 2013; Moreno et al., 2014). It has been reported that diploid and triploid Mulberry (Morus Alba L.) plants grown under drought stress conditions are more tolerant than diploids under drought stress conditions because triploid plants have high abscisic acid (ABA) levels (Liu et al., 2021).

Zhu et al. (2018), reported that under salt stress conditions, plants grafted on tetraploid rootstocks were less affected by salt stress because they had higher biomass and photosynthesis rates than plants grafted on diploid rootstocks. In addition, by grafting diploid plants onto tetraploid plants and teraploid plants onto diploid plants, they obtained information that the higher salt tolerance of tetraploid watermelon plants is determined by the root, not the leaf.

In plant breeding today, colchicine is one of the most commonly used chemicals for chromosome doubling. It is a powerful alkaloid poison derived from the roots of autumn crocus (*Colchicum autumnale* L.). It is colorless, soluble in alcohol, chloroform and cold water; it does not dissolve in hot water or ether. Its chemical formula is shown as C22H25O6. Colchicine prevents the formation of spindle fibers during metaphase of mitosis in the cells of tissues to which it is applied, which allows the replicated chromosomes to be pulled to the poles, doubling the number of chromosomes (Köksal, 1999). However, the use of alternative compounds to Colchicine has been investigated due to its high mutagenicity, relatively high harm to the environment, and its high cost. In this context, it has been revealed that oryzalin, trifluralin, amiprophos-methyl, N2O can be used successfully in in vivo and in vitro studies for the same purpose (Grzebelus & Adamus, 2004).

Although colchicine is effective in producing polyploid plants, it is a highly toxic, carcinogenic compound (Morejohn et al., 1987). Orizalin, trifluralin, amiprofos-methyl1 (APM) and caffeine have been used as alternatives to colchicine. Oryzalin has high rates of polyploidy and plant survival and low toxicity to organisms (Dhooghe et al., 2011).

Watermelon is an annual plant belonging to the *Cucurbitaceae* family. It is one of the important economic crops, widely grown for its sweet fruit. Typically, diploid watermelons (2n = 2× = 22) are more common in nature, while autopolyploids enable triploid watermelons (3n = 3× = 33) and tetraploid watermelons (4n = 4× = 44) (Blakeslee & Avery, 1937). The triploid watermelon is a hybrid of a diploid and a tetraploid watermelon, and it is known for its seedless fruit. Autopolyploids have diverse DNA contents, high secondary metabolite organisms, large tissues and organs, improved yield, and high tolerance to both biotic and abiotic stresses (Godfree et al., 2017; Soltis et al., 2016). In tetraploid watermelons, the leaves are broad, the leech and rind are thicker, and the pistil and stamen flower organs are larger. In addition, tetraploid watermelon is richer in chlorophyll, lycopene, fructose, and glucose than diploid watermelon (Jaskani, Kwon, & Kim, 2005; Wenge Liu et al., 2010)); and more resistant to salt stress than diploid watermelon. (Feng et al., 2022). However, the low frequency of autopolyploids and uncertainty in monitoring and selection limit its application to commercial breeding.

Because autoploids contain quite a lot of 2 homologous chromosomes and yield monovalent and polyvalent chromosomes after meiosis occurs, it is sometimes different in diploids where divalent chromosomes are formed (Acquaah, 2007). As an example across meiosis, autotetraploids can form divalent, tetravalent and monovalent species. Polyploid fruits and vegetables are produced artificially by first developing tetraploids and then crossing them with diploid plants. The seedless feature of triploids may provide a marketing advantage. The successful use of Datura tetraploidy created with colchicine by Blakeslee and Avery in 1937 encourages the artificial creation of polyploids (Blakeslee & Avery, 1937). Colchicine was applied to newly emerged diploid seedlings to produce tetraploid parents (Suying et al., 1995). Tetraploid pure lines can also be propagated by seed and tissue culture (Krug et al., 2005). Polyploids are created, observation and selection become more important. It has been possible to determine the ploidy level of watermelon using a variety of approaches. Morphological characterization is the most intuitive method. The length and width of leaves, the size of ovaries, the size of male flower petals, and the size of pollen sacs can be used to identify species, according to research (Compton et al., 1996; Rhodes & Zhang, 1999). There is a positive correlation between the number of chloroplasts in watermelon leaf guard cells and plant ploidy, which can also be used as a rapid and effective method (Sari et al., 1999). Chromosome counting is also used to determine ploidy in cells that do not divide, such as leaves, but the small size of watermelon chromosomes makes it difficult to be accurate (Fahleson et al., 1988). Watermelon tetraploid reproduction therefore requires an effective and reliable ploidy measurement technique. An increase or decrease in chromosome number corresponds to an increase or decrease in nuclear DNA content (Arumuganathan & Earle, 1991). It can be applied to tissues grown in the field or greenhouse. Although there is a limited choice of organization, FCM is better at detecting young tissues with poor cell division. In addition, traditional flow cytometers are expensive and rarely used in routine laboratories. A mature method for detecting gene expression and analyzing copy number is qPCR (Fleige & Pfaffl, 2006; Rubio-Piña et al., 2016). Some studies on polyplidasis in watermelon are summarized below.

Lower & Johnson (1969), recommended applying 0.3% colchicine to the growth points of seedlings as 2 drops in 24 hours, 8 drops in 52 hours, 8 drops in 80 hours or 6-24 hours before planting to obtain tetraploid watermelon lines. They carried out a study by dipping it in 0.1%-0.2% colchicine solution for hours (6 hours apart). In the study, Charleston Gray 133 and Princeton varieties were used, and as a result of the research, it was determined that the Princeton variety responded better to tetraploidy than Charleston Gray 133. Researchers found that the application of 8 drops of colchicine (at 52 and 80 hours) provided 6% tetraploid induction in the Princeton variety; They reported that this rate was 4% in the C. Gray variety. Application of two drops (24 hours) of colchicine gave no results on C. Gray variety; It was determined that the Princeton variety produced tetraploidy at a level of 3%. It was determined that keeping the seeds in 0.1% colchicine solution for 24 hours or 0.2% colchicine solution for 18 hours resulted in 0-20% tetraploidy. Researchers have stated that the seed dipping method is more effective and easier than the method of dripping onto the growing tip.

Li ve ark. (2002), investigated the effect of colchicine application to obtain tetraploid plants in watermelon line number 2608 (YPW) with yellow fruit skin. Colchicine at a dose of 0.2% was applied to the seedlings growing from the seeds sown in February under greenhouse conditions, at the stage of 1-2 true leaves. In the study examining the mutations and physiological changes caused by colchicine in organs; Morphological, microscopic and hybridization effects were studied with different ploidy determination methods. As a result of the research, the tetraploidy rate was found to be 12.4%, and triploid plants were developed by hybridizing tetraploids with diploids.

Koh (2002), applied the antimitotic factors colchicine (0.05, 0.10, 0.20 and 0.50) and oryzaline (5, 15, 30 and 60 μM) to young seedlings in a study aimed at developing tetraploid watermelon production methods. In the study, the effects of different antimitotic factors applied in early development periods on the level of tetraploidization and the differences between diploid and tetraploid were investigated. When 0.2% colchicine and 60 μM oryzalin were applied to young seedlings for 48 hours, shoot development was weakened in more than 50% of the plants. reported that it was as high as the application. When diploid and tetraploid plants are compared; It was determined that there were differences in the number of chloroplasts in the guard cells, anther shape, pollen amount in the male flower and leaf shapes. Analysis results obtained from flow cytometry showed that many tetraploid plants are mixoploid. He reported that the amount of seeds per fruit in tetraploid plants is approximately 1/10 less than in diploids, and most of them produce empty seeds.

The high hormone content and high antioxidant activities of tetraploid rootstocks are higher than diploids (Kaseb et al., 2020). The tetraploid watermelon rootstock USVL-360 gave the same yield as commercial rootstocks and provided resistance against root-knot nematode (Levi et al., 2014).

Jaskani, Kwon, & Kin (2005), investigated the promote ploidy change (especially to obtain tetraploid), they applied colchicine, an antimitotic agent, to watermelons in in vitro culture. As a method in the research; It was chosen to culture cross sections of cotyledon explant, embryogenic tip of the seed, epicotyl and hypocotyl in MS medium containing BA (1µM) and colchicine (0.01%, 0.05% and 0.1%). After 4-7 days, the explants were cultured in colchicine-free medium. As a result of the research; It was stated that colchicine had a negative effect on in vitro regeneration, however, it was determined that the hypocotyl parts of the seedlings formed maximum callus in 0.01% colchicine. It was determined that cotyledon explants formed more and faster shoots than those cultured in colchicine at a concentration of 0.01% for 4 days. Maximum root formation and number of roots were observed in embryogenic explants. As a result, the cotyledon and embryogenic tips of the explants were determined to be optimum at low colchicine concentration (0.01%) in watermelon regeneration.

Rhodes & Zhang (1999), reported that controlled pollination plays a fundamental role in hybrid watermelon seed production and that the use of male sterility requires great effort. They reported that successful seed production depends on good culture conditions and timely harvest, and the amount of triploid seed production is higher than the amount of diploid hybrid seed production. They stated that the development of tetraploid parents increased more with the use of chromosome doubling factors other than colchicine and tissue culture. They stated that triploid hybrids, with two chromosomes from the female parent and one chromosome from the paternal parent, can offer the consumer more varieties and new strategies such as resistance to diseases. New triploids can meet market demands because they enable rapid variety development in response to a new disease problem.

Germination of triploid watermelon seeds is quite difficult because the thickness of the tetraploid female seed coat passes into the triploid embryo and the embryo is weak (Phat et al., 2015). The cost of triploid watermelon seeds is higher than diploid seed varieties in proportion to the lower number of seeds obtained from the tetraploid female plant. Triploid seeds are expensive because the formation process of triploid seeds is long and labor costs are high. In general, the germination percentage of tetraploid seeds is low because the embryo sizes are smaller (Nerson et al., 2022). The germination rate of a triploid seed is generally between 60% and 80%. Unlike triploid seed, the germination rate of diploid seed is 95% with its homogeneity and increased seedling strength. Thus, triploid watermelons are often provided at an additional cost to the grower (Grange et al., 2003). Polyploid watermelons have also been found to be resistant to watermelon bacterial fruit spot disease (Acidivorax avenae subsp. citrulli) and nematodes (Montalvo & Esnard, 1994).

Genetic and morphological diversity are created by polyploidy in plant morphology. This study was designed to distinguish tetraploids from diploids morphologically. Tetrapoloids were used to distinguish diploids from triploids morphologically. A number of morphological parameters were observed in this study, including chloroplast number, pollen colpis, seedling mortality (%), fruit set (%), fruit weight (kg), TSS, peel thickness, and number of seeds per fruit. Chloroplasts per guard cell were higher in Tetrapoloid-2 (9.0), shell thickness was higher (14.7 mm), and fruit set (%) was higher in diploids (94.3) (Ahmad et al., 2013). Perkins-Veazie et al. (2006), report that triploid varieties typically have higher overall lycopene content than diploid varieties.

According to Salsabila et al. (2021), ISSR markers are an effective and successful method for determining the ploidy level in horticultural plants, especially watermelon. Genetic variation analysis using the PCR-ISSR method reported that ISSR BI, B3, B5 and CBTC 1 molecular markers could be used to distinguish ploidy from tested watermelons.

In a study conducted by Aydın et al. (2022), the rootstock potential of autotetraploid and allotetraploid citrullus genotypes for watermelon was investigated under hydroponic conditions. The results showed that the choice of rootstock genotype significantly affected plant growth and physiological parameters. Watermelon plants grafted on N7-4T tetraploid rootstock had the longest plant stems (62.67 cm), while plants on autotetraploid Calhounn Gray had the shortest stems (14.33 cm). The grafting combinations N7-4T/CT and CN7-5T/CT resulted in the highest shoot wet and dry weights. Similarly, plants grafted on tetraploid rootstocks had higher root wet and dry weights compared to those grafted on diploid and commercial rootstocks. The highest root wet and dry weights were observed in plants grafted on autotetraploid N5-4T and allotetraploid CN7-5T. Root traits and leaf nutrient contents (N, P, K, and Ca) were also significantly influenced by the rootstock genotypes. Overall, the study suggests that polyploidy-derived citrullus tetraploid genotypes can be a promising alternative source of rootstocks for watermelon cultivation.

Lower & Johnson (2002), to obtain tetraploid watermelon lines, apply 0.3% colchicine to the growth points of the seedlings as 2 drops in 24 hours, 8 drops in 52 hours, 8 drops in 80 hours or 0.1% - 0.2% to the seeds for 6-24 hours (6 hour intervals) before planting. They conducted a study by dipping it in colchicine solution. In the study, Charleston Gray 133 and Princeton varieties were used, and as a result of the research, it was determined that the Princeton variety responded better to tetraploidy than Charleston Gray 133. Researchers found that the application of 8 drops of colchicine (at 52 and 80 hours) provided 6% tetraploid induction in the Princeton variety; They reported that this rate was 4% in the C. Gray variety. Application of two drops (24 hours) of colchicine did not cause any response in C. Gray variety; It was determined that the Princeton variety produced tetraploidy at the level of 3%. It has been determined that keeping the seeds in 0.1% colchicine solution for 24 hours or 0.2% colchicine solution for 18 hours creates tetraploidy at a rate of 0-20%. Researchers have stated that the seed dipping method is more effective than the method of dripping onto the growing tip.

Aydin & Yetişir (2023), determined the ploidy levels of polyploidy-induced plants by stomatal observations and flow cytometry analysis by applying 0.05% colchicine to the seedlings at the first true leaf stage. Flow cytometry and stomatal observations revealed 11% and 3% polyploidy formation rates, respectively, for developing polyploid watermelon rootstocks. According to flow cytometry results, 22 polyploidy genotypes were identified. The hydroponic growth performance of auto- (12) and allotetraploid (10) citrus genotypes was compared with that of diploids, commercial rootstocks (RS841, 'Argentario') and watermelon cultivars ('Crimson Tide'). In hydroponic culture, putative tetraploids and diploid controls were grown for 21 days to determine their vegetative growth performance. Compared to diploid controls, autotetraploids and allotetraploids increased their biomass by 100% and 156%, respectively.

Zhang et al. (2014), colchicine was applied to cotyledons of a diploid mini watermelon (A7) for different periods of time. Morphology, chloroplast compartment determination in stomatal protection, and variability cytometry analysis can be used to identify autotetraploid plants. A stable autotetraploid material was observed. It was possible to obtain results under different treatments of tetraploid watermelons, and the highest tetraploid induction method of 25% was used. Explants were cultured on MS medium containing 0.1% (w/v) colchicine for 72 hours after cutting the proximal cotyledons on day 7 after sowing. There was a 62.5% incidental shoot induction rate and a 3.6 impact multiplicity.

Kaur et al. (2018), In producing hypothetical tetraploids, California Sweet had the highest efficiency rate (6.12%). A reverse hypocotyl and shoot tip method and colchicine concentrations of 0.2% and 0.3% were relatively more effective than other colchicine methods and concentrations for the generation of tetraploids. The highest frequency of putative tetraploids (11.55%) was recorded with the (0.3% colchicine) reverse hypocotyl method.

Şimşek et al. (2013), pre-selection of diploid and tetraploid watermelon plants in the population can be done by evaluating their leaf and flower characteristics. Plants that are morphologically different from diploid plants selected by preliminary selection should be selected again according to stomatal size and density and the number of chloroplasts in guard cells by cytological analysis. The distinction between tetraploid and mixploid plants can be achieved by selfing the female flower and ensuring fruit set. Since myxoploid plants do not produce fruit, these plants are lost later. Precise identification of tetraploid plants can be made by chromosome counting and flow cytometric analysis. However, in cases where there is no laboratory infrastructure, being able to move on to the next generation with morphological and cytological evaluations and fruit set makes tetraploid line development in watermelon and selection at the M1 stage easy, feasible and economical.

One of the most important mechanisms of adaptation and speciation in plants is polyploidy, which has been extensively studied over the last century. New crop species can be evolved using polyploidy breeding. Larger fruits or other parts tend to result in higher yields and prices for farmers. As a result, biotic and abiotic stress resistance is improved. Polyploidy has also proven to be of great importance for humans due to the fact that many of the most important crop species are polyploid.

REFERENCES

Abel, S., & Becker, H. C. (2007). The effect of autopolyploidy on biomass production in homozygous lines of Brassica rapa and Brassica oleracea. *Plant Breeding*, *126*(6), 642–643. https://doi.org/10.1111/J.1439-0523.2007.01405.X

Adams, K. L., & Wendel, J. F. (2005). Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology*, *8*(2), 135–141. https://doi.org/10.1016/J.PBI.2005.01.001

Ahmad, I., Hussain, T., Nafees, M., Jamil, M., Ashraf, I., Fakhar-u-Zaman Akhtar, M., Iqbal, M., Rafay, M., Ruby, T., & Ali, L. (2013). Morphological Dissimilarity Between Tetrapoloid and Diploid Watermelon (Citrullus lanatus Thunb.). *World Applied Sciences Journal*, *21*(6), 858–861. https://doi.org/10.5829/idosi.wasj.2013.21.6.169

Arumuganathan, K., & Earle, E. D. (1991). Estimation of nuclear DNA content of plants by flow cytometry. *Plant Molecular Biology Reporter*, *9*(4), 415. https://doi.org/10.1007/BF02672017/METRICS

Aydin, A., & Yetişir, H. (2023). A comparative study of morphological characteristics in diploid and tetraploid (auto and allotetraploids) Citrullus genotypes. *Folia Horticulturae*, *35*(1), 33–48. https://doi.org/10.2478/FHORT-2023-0003

Aydın, A., Yetişir, H., Başak, H., Turan, M., & Tuna, M. (2022). Rootstock potential of auto and Allotetraploid Citron [Citrullus lanatus var. citroides (L. H. Bailey) Mansf.] for Watermelon [Citrullus lanatus var lanatus (Thunb.) Matsum. & Nakai] under hydroponic conditions: plant growth and some physiological characteristics. *International Journal of Agriculture Environment and Food Sciences*, *6*(4), 648–659. https://doi.org/10.31015/JAEFS.2022.4.20

Balal, R. M., Shahid, M. A., Vincent, C., Zotarelli, L., Liu, G., Mattson, N. S., Rathinasabapathi, B., Martínez-Nicolas, J. J., & Garcia-Sanchez, F. (2017). Kinnow mandarin plants grafted on tetraploid rootstocks are more tolerant to Cr-toxicity than those grafted on its diploids one. *Environmental and Experimental Botany*, *140*, 8–18. https://doi.org/10.1016/J.ENVEXPBOT.2017.05.011

Blakeslee, A. F., & Avery, A. G. (1937). Methods of inducing doubling of chromosomes in plants: By treatment with colchicine. *Journal of Heredity*, *28*(12), 393–411. https://doi.org/10.1093/OXFORDJOURNALS.JHERED.A104294/2/28-12-393.PDF.GIF

Bowers, J. E., Chapman, B. A., Rong, J., & Paterson, A. H. (2003). Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature*, *422*(6930), 433–438. https://doi.org/10.1038/NATURE01521

Briggs, D. E. (1978). Barley improvement. *Barley*, 445–480. https://doi.org/10.1007/978-94-009-5715-2_12

Chao, D. Y., Dilkes, B., Luo, H., Douglas, A., Yakubova, E., Lahner, B., & Salt, D. E. (2013). Polyploids exhibit higher potassium uptake and salinity tolerance in Arabidopsis. *Science*, *341*(6146), 658–659. https://doi.org/10.1126/SCIENCE.1240561/SUPPL_FILE/DATABASE.S4.XLSX

Chen, Z. J. (2007). Genetic and Epigenetic Mechanisms for Gene Expression and Phenotypic Variation in Plant Polyploids. *Http://Dx.Doi.Org/10.1146/Annurev.Arplant.58.032806.103835*, *58*, 377–406. https://doi.org/10.1146/ANNUREV.ARPLANT.58.032806.103835

Cohen, H., & Tel-Zur, N. (2012). Morphological changes and self-incompatibility breakdown associated with autopolyploidization in Hylocereus species (Cactaceae). *Euphytica*, *184*(3), 345–354. https://doi.org/10.1007/S10681-011-0536-5/FIGURES/4

Compton, M. E., Gray, D. J., & Elmstrom, G. W. (1996). Identification of tetraploid regenerants from cotyledons of diploid watermelon cultured in vitro. *Euphytica*, *87*(3), 165–172. https://doi.org/10.1007/BF00023744/METRICS

Corso, M., Vannozzi, A., Ziliotto, F., Zouine, M., Maza, E., Nicolato, T., Vitulo, N., Meggio, F., Valle, G., Bouzayen, M., Müller, M., Munné-Bosch, S., Lucchin, M., & Bonghi, C. (2016). Grapevine rootstocks differentially affect the rate of ripening and modulate auxin-related genes in cabernet sauvignon berries. *Frontiers in Plant Science*, *7*(FEB2016), 69. https://doi.org/10.3389/FPLS.2016.00069/BIBTEX del Pozo, J. C., & Ramirez-Parra, E. (2014). Deciphering the molecular bases for drought tolerance in A rabidopsis autotetraploids. *Plant, Cell & Environment*, *37*(12), 2722–2737.

Dhooghe, E., van Laere, K., Eeckhaut, T., Leus, L., & van Huylenbroeck, J. (2011). Mitotic chromosome doubling of plant tissues in vitro. *Plant Cell, Tissue and Organ Culture*, *104*(3), 359–373. https://doi.org/10.1007/S11240-010-9786-5

Doyle, J. J., & Coate, J. E. (2018). Polyploidy, the Nucleotype, and Novelty: The Impact of Genome Doubling on the Biology of the Cell. *International Journal of Plant Sciences*, *180*(1), 1–52. https://doi.org/10.1086/700636

Doyle, J. J., & Egan, A. N. (2010). Dating the origins of polyploidy events. *New Phytologist*, *186*(1), 73–85. https://doi.org/10.1111/J.1469-8137.2009.03118.X

Doyle, J. J., Flagel, L. E., Paterson, A. H., Rapp, R. A., Soltis, D. E., Soltis, P. S., & Wendel, J. F. (2008). Evolutionary Genetics of Genome Merger and Doubling in Plants. *Http://Dx.Doi.Org/10.1146/Annurev.Genet.42.110807.091524*, *42*, 443–461. https://doi.org/10.1146/ANNUREV.GENET.42.110807.091524

Fahleson, J., Dixelius, J., Sundberg, E., & Glimelius, K. (1988). Correlation between flow cytometric determination of nuclear DNA content and chromosome number in somatic hybrids within Brassicaceae. *Plant Cell Reports*, *7*(1), 74–77. https://doi.org/10.1007/BF00272983/METRICS

Feng, Z., Bi, Z., Fu, D., Feng, L., Min, D., Bi, C., & Huang, H. (2022). A Comparative Study of Morphology, Photosynthetic Physiology, and Proteome between Diploid and Tetraploid Watermelon (Citrullus lanatus L.). *Bioengineering*, *9*(12), 746. https://doi.org/10.3390/BIOENGINEERING9120746/S1

Fleige, S., & Pfaffl, M. W. (2006). RNA integrity and the effect on the real-time qRT-PCR performance. *Molecular Aspects of Medicine*, *27*(2–3), 126–139. https://doi.org/10.1016/J.MAM.2005.12.003

Godfree, R. C., Marshall, D. J., Young, A. G., Miller, C. H., & Mathews, S. (2017). Empirical evidence of fixed and homeostatic patterns of polyploid advantage in a keystone grass exposed to drought and heat stress. *Royal Society Open Science*, *4*(11). https://doi.org/10.1098/RSOS.170934

Grange, S., Leskovar, D. I., Pike, L. M., & Cobb, B. G. (2003). Seedcoat structure and oxygen-enhanced environments affect germination of triploid watermelon. *Journal of the American Society for Horticultural Science*, *128*(2), 253–259. https://doi.org/10.21273/JASHS.128.2.0253

Gregory, P. J., Atkinson, C. J., Bengough, A. G., Else, M. A., Fernández-Fernández, F., Harrison, R. J., & Schmidt, S. (2013). Contributions of roots and rootstocks to sustainable, intensified crop production. *Journal of Experimental Botany*, *64*(5), 1209–1222.

Grzebelus, E., & Adamus, A. (2004). Effect of anti-mitotic agents on development and genome doubling of gynogenic onion (Allium cepa L.) embryos. *Plant Science*, *167*(3), 569–574. https://doi.org/10.1016/J.PLANTSCI.2004.05.001

Harlan, J. R. (1995). The living fields: our agricultural heritage. *Cambridge University Press.* https://doi.org/10.3/JQUERY-UI.JS

Hayward, M. D., Romagosa, I., & Bosemark, N. O. (1993). *Plant breeding : principles and prospects*. 550.

Jaskani, M. J., Kwon, S. W., & Kim, D. H. (2005). Comparative study on vegetative, reproductive and qualitative traits of seven diploid and tetraploid watermelon lines. *Euphytica*, *145*, 259–268. https://doi.org/10.1007/s10681-005-1644-x

Jaskani, M. J., Kwon, S. W., & Kin, H. (2005). FLOW CYTOMETRY OF DNA CONTENTS OF COLCHICINE TREATED WATERMELON AS A PLOIDY SCREENING METHOD AT M1 STAGE. *Pak. J. Bot*, *37*(3), 685–696.

Kaseb, M., Umer, M. J., Gebremeskel, H., Mahmud, E., Diao, W., Yuan, P., Zhu, H., Zhao, S., Lu, X., & He, N. (2020). Comparative Physiological and Biochemical Mechanisms in Di, Tri, and Tetraploid Watermelon (Citrullus lanatus L.) Grafted by Branches. *Preprints (Www.Preprints.Org)*. https://doi.org/10.20944

Kaur, B., Singh, R., & Singh, D. (2018). Screening of tetraploidy induction methods using anti-microtubule agent colchicine in watermelon

(Citrullus lanatus (Thunb.) Matsum & Nakai). *Vegetable Science*, *45*(02), 166–172. https://doi.org/10.61180/VBW0YP83

King, S. R., Davis, A. R., Zhang, X., & Crosby, K. (2010). Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. *Scientia Horticulturae*, *127*, 106–111. https://doi.org/10.1016/j.scienta.2010.08.001

Klug, W. S., Cummings, M. R., Spencer, C. A., & Öner, Cihan. (2011). Genetik kavramlar. *Https://Acikbilim.Yok.Gov.Tr/Handle/20.500.12812/156200*.

Koh, G.-C. (2002). Tetraploid production of Moodeungsan watermelon. *Journal-Korean Socıety For Hortıcultural Scıence*, *43*(6), 671–676.

Köksal, N. (1999). Haploid kavun bitkilerinde in vitro ve in vivo yöntemlerle diploidizasyon. *Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Bahçe Bitkileri Ana Bilim Dalı, Yüksek Lisans, Adana, 131 s.* https://acikbilim.yok.gov.tr/handle/20.500.12812/156200

Krug, M. G. Z., Stipp, L. C. L., Rodriguez, A. P. M., & Mendes, B. M. J. (2005). In vitro organogenesis in watermelon cotyledons. *Pesquisa Agropecuária Brasileira*, *40*(9), 861–865. https://doi.org/10.1590/S0100-204X2005000900004

Levi, A., Thies, J. A., Wechter, P. W., Farnham, M., Weng, Y., & Hassell, R. (2014). USVL-360, a Novel Watermelon Tetraploid Germplasm Line. *HortScience*, *49*(3), 354–357. https://doi.org/10.21273/HORTSCI.49.3.354

Li, X., Yu, E., Fan, C., Zhang, C., Fu, T., & Zhou, Y. (2012). Developmental, cytological and transcriptional analysis of autotetraploid Arabidopsis. *Planta*, *236*(2), 579–596.

Liu, H., Sun, H., Bao, L., Han, S., Hui, T., Zhang, R., Zhang, M., Su, C., Qian, Y., & Jiao, F. (2021). Secondary Metabolism and Hormone Response Reveal the Molecular Mechanism of Triploid Mulberry (*Morus Alba L.*) Trees Against Drought. *Frontiers in Plant Science, 12*, 720452–720452. https://doi.org/10.3389/FPLS.2021.720452

Lı, L., He, Y., Lu, K., (No.3), 8-11. (2002). Chemical Induction Mutation In Yellow Peel Watermelon (Citrullus lanatus) And its Application To Tetraploid Watermelon Breeding. *Chne Vegetables*, *3,* 8–11.

Lower, R. L., & Johnson, K. W. (1969). Observations on sterility of induced auto-tetraploid watermelons. *J. Amer. Soc. Hort. Sci*, *94*, 367–369. https://eurekamag.com/research/014/583/014583568.php

Lower, R. L., & Johnson, K. W. (2022). Observations on Sterility of Induced Autotetraploid Watermelons1. *Journal of the American Society for Horticultural Science*, *94*(4), 367–369. https://doi.org/10.21273/JASHS.94.4.367

Montalvo, A. E., & Esnard, J. (1994). Reaction of Ten Cultivars of Watermelon (Citrullus lanatus) to a Puerto Rican population of Meloidogyne incognita. *Journal of Nematology*, *26*(4S), 640. /pmc/articles/PMC2619540/?report=abstract

Morejohn, L. C., Bureau, T. E., Molè-Bajer, J., Bajer, A. S., & Fosket, D. E. (1987). Oryzalin, a dinitroaniline herbicide, binds to plant tubulin and inhibits microtubule polymerization in vitro. *Planta*, *172*(2), 252–264. https://doi.org/10.1007/BF00394595/METRICS

Moreno, M. J. C., Hevin, C., Ollat, N., & Cookson, S. J. (2014). Developments at the graft interface in homo- and hetero-grafts: Gene expression and histological changes during the first month after grafting. *Plant Signaling & Behavior*, *9*(6), 2997–3008. https://doi.org/10.4161/PSB.28852

Nerson, H., Paris, H. S., Karchi, Z., & Sachs, M. (2022). Seed Treatments for Improved Germination of Tetraploid Watermelon. *HortScience*, *20*(5), 897–899. https://doi.org/10.21273/HORTSCI.20.5.897

Otto, S. P., & Whitton, J. (2003). Polyploİd Incİdence and Evolution. *Https://Doi.Org/10.1146/Annurev.Genet.34.1.401*, *34*, 401–437. https://doi.org/10.1146/ANNUREV.GENET.34.1.401

Perkins-Veazie, P., Collins, J. K., Davis, A. R., & Roberts, W. (2006). Carotenoid Content of 50 Watermelon Cultivars. *Journal of Agricultural and Food Chemistry*, *54*(7), 2593–2597. https://doi.org/10.1021/jf052066p

Phat, P., Sheikh, S., Lim, J. H., Kim, T. B., Seong, M. H., Chon, H. G., Shin, Y. K., Song, Y. J., & Noh, J. (2015). Enhancement of Seed Germination and Uniformity in Triploid Watermelon (Citrullus lanatus (Thunb.) Matsum. and Nakai). *Horticultural Science &*

Technology, *33*(6), 932–940. https://doi.org/10.7235/HORT.2015.14193

Rejeb, R. ben, & Benbadis, A. (1989). Fertile allotetraploid from the cross betweenPhaseolus coccineus L. andPhaseolus acutifolius A. Gray. *Plant Cell Reports 1989 8:3*, *8*(3), 178–181. https://doi.org/10.1007/BF00716835

Rhodes, B., & Zhang, X. (1999). Hybrid Seed Production in Watermelon. *Journal of New Seeds*, *1*(3–4), 69–88. https://doi.org/10.1300/J153V01N03_03

Rubio-Piña, J., Quiroz-Moreno, A., & Sánchez-Teyer, L. F. (2016). A quantitative PCR approach for determining the ribosomal DNA copy number in the genome of Agave tequila Weber. *Electronic Journal of Biotechnology*, *22*, 9–15. https://doi.org/10.1016/J.EJBT.2016.05.002

Ruiz, M., Quiñones, A., Martínez-Cuenca, M. R., Aleza, P., Morillon, R., Navarro, L., Primo-Millo, E., & Martínez-Alcántara, B. (2016). Tetraploidy enhances the ability to exclude chloride from leaves in carrizo citrange seedlings. *Journal of Plant Physiology*, *205*, 1–10. https://doi.org/10.1016/J.JPLPH.2016.08.002

Saleh, B., Allario, T., Dambier, D., Ollitrault, P., & Morillon, R. (2008). Tetraploid citrus rootstocks are more tolerant to salt stress than diploid. *Comptes Rendus Biologies*, *331*(9), 703–710. https://doi.org/10.1016/J.CRVI.2008.06.007

Salsabila, A. J., Subiastuti, A. S., & Daryono, B. S. (2021). *Identification of ISSR-based Molecular Markers Associated with Ploidy Level of Orange Watermelon (Citrullus lanatus (Thunb.) Matsum & Nakai)*. https://doi.org/10.22146/jtbb.61858

Sari, N., Abak, K., & Pitrat, M. (1999). Comparison of ploidy level screening methods in watermelon: Citrullus lanatus (Thunb.) Matsum. and Nakai. *Scientia Horticulturae*, *82*(3–4), 265–277. https://doi.org/10.1016/S0304-4238(99)00077-1

Sattler, M. C., Carvalho, C. R., & Clarindo, W. R. (2016). The polyploidy and its key role in plant breeding. *Planta*, *243*(2), 281–296.

Şimşek, İ., Göçmen, M., Sari, N., Tarım AŞ, A., Üniversitesi, Ç., Fakültesi, Z., & Bitkileri Bölümü, B. (2013). DİPLOİD VE TETRAPLOİD KARPUZ BİTKİLERİNDE MORFOLOJİK VE SİTOLOJİK

FARKLILIKLARIN BELİRLENMESİ. *Batı Akdeniz Tarımsal Araştırma Enstitüsü Derim Dergisi*, *30*(1), 1–14.

Soltis, D. E., Misra, B. B., Shan, S., Chen, S., & Soltis, P. S. (2016). Polyploidy and the proteome. *Biochimica et Biophysica Acta - Proteins and Proteomics*, *1864*(8), 896–907. https://doi.org/10.1016/J.BBAPAP.2016.03.010

Suying, T., Xiuqiang, H., Jiwei, L., & Wenge, L. (1995). RAISING THE FREQUENCY OF INDUCING TETRAPLOID WATERMELON BY TREATING OF COLCHICINE. *Acta Horticulturae*, *402*, 18–22. https://doi.org/10.17660/ACTAHORTIC.1995.402.2

Tester, M., & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science (New York, N.Y.)*, *327*(5967), 818–822. https://doi.org/10.1126/SCIENCE.1183700

Wendel, J. F. (2000). Genome evolution in polyploids. *Plant Molecular Evolution*, 225–249. https://doi.org/10.1007/978-94-011-4221-2_12

Wenge Liu, Shengjie Zhao, Zhiqiang Cheng, Xueshan Wan, Zhihong Yan, & King, S. R. (2010). LYCOPENE AND CITRULLINE CONTENTS IN WATERMELON (CITRULLUS LANATUS) FRUIT WITH DIFFERENT PLOIDY AND CHANGES DURING FRUIT DEVELOPMENT. *Acta Horticulturae*, *871*, 543–550. https://doi.org/10.17660/ACTAHORTIC.2010.871.75

Yao, H., Kato, A., Mooney, B., & Birchler, J. A. (2011). Phenotypic and gene expression analyses of a ploidy series of maize inbred Oh43. *Plant Molecular Biology*, *75*(3), 237–251. https://doi.org/10.1007/S11103-010-9722-4/TABLES/4

Zhang, N., Bao, Y., Xie, Z., Huang, X., Sun, Y., Feng, G., Zeng, H., Ren, J., Li, Y., & Xiong, J. (2019). Efficient characterization of tetraploid watermelon. *Plants*, *8*(10), 419.

Zhang, N., Ren, J., Cheng, W. S., Zeng, H. X., Shi, X. F., Li, Y. H., & Sun, Y. H. (2014). In Vitro Induction and Identification of Tetraploid in Watermelon [Citrullus lanatus (Thunb.) Matsum and Nakai]. *Applied Mechanics and Materials*, *675–677*, 1079–1086. https://doi.org/10.4028/WWW.SCIENTIFIC.NET/AMM.675-677.1079

Zhao, L., Liu, A., Song, T., Jin, Y., Xu, X., Gao, Y., Ye, X., & Qi, H. (2018). Transcriptome analysis reveals the effects of grafting on sugar and α-linolenic acid metabolisms in fruits of cucumber with two different rootstocks. *Plant Physiology and Biochemistry*, *130*, 289–302. https://doi.org/10.1016/J.PLAPHY.2018.07.008

Zhu, H., Zhao, S., Lu, X., He, N., Gao, L., Dou, J., Bie, Z., & Liu, W. (2018). Genome duplication improves the resistance of watermelon root to salt stress. *Plant Physiology and Biochemistry*, *133*, 11–21. https://doi.org/10.1016/J.PLAPHY.2018.10.019

CHAPTER 18

OVERVIEW OF DETECTION OF PESTICIDES WITH COLORIMETRIC METHODS

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INTRODUCTION

The world is on the brink of a food crisis in the near future as a result of industrialization and global warming. This will lead to agricultural areas becoming non-functional, a decline in freshwater supplies, and an increase in population. Agriculture, being the first stage of the food production chain, plays a pivotal role in the creation of raw materials, making it the most crucial aspect of the process. Increased agricultural product availability directly correlates with higher levels of food production. Agricultural production is influenced by several elements such as climate, soil qualities, and agricultural pests. Agricultural pests result in a reduction in crop output and leave the farmer in a state of helplessness. Pesticides, now used as a solution, safeguard agricultural crops against pests, although their poisonous properties represent a threat to human well-being and contribute to environmental contamination.

Precise analysis of chemical residues in plants, as well as the measurement of their presence in soil and water resources, is an essential need in agricultural practices, alongside dose adjustments in pesticide application. Given its potential impact on commerce and public health, this stage is crucial. The recent interruption in the export of vegetables and fruits owing to pesticide residues highlights the need of doing precise quantity analysis from an economic standpoint. However, it is crucial to examine the levels of exposure and accumulation of pesticides, since they might have harmful effects on both people and other organisms. Pesticides, particularly when present in soil and clean water supplies, may lead to irreversible environmental catastrophes.

1.Pesticides

A pesticide is a chemical ingredient or mixture that is used to stay away from, eradicate, or manage pests, including insects, fungus, rodents, or unwanted plant species, which may cause harm during the cultivation and storage of crops. Pesticide is a broad phrase that includes several compounds, such as insecticides, herbicides, fungicides, and rodenticides, which are used to eradicate certain pests. Pesticides are classified according to their origins, which might be either chemical or biopesticides. Biological pesticides demonstrate host selectivity, as stated by Abubakar et al. (Abubakar et al., 2019) .The global consumption of pesticides in 2019 approximated 4.19 million metric tons. According to Fernández, China was the foremost user of pesticides, using 1.76 million metric tons. The United States followed with 408 thousand tons, Brazil with 377 thousand tons, and Argentina with 204 thousand tons. The World Health Organization (WHO) has disclosed that pesticide usage in Southeast Asia is growing on a yearly basis. Consumers of pesticides in the area, including Cambodia, Laos, and Vietnam, account for around 20% of developing countries (Pathak et al., 2022).

1.1 Classification of Pesticides

Pesticides are categorized according to their intended use. Based on its source, it may be classified into two categories: natural (biological) and synthetic. Cllasificatiob scheme is given at Figure 1.

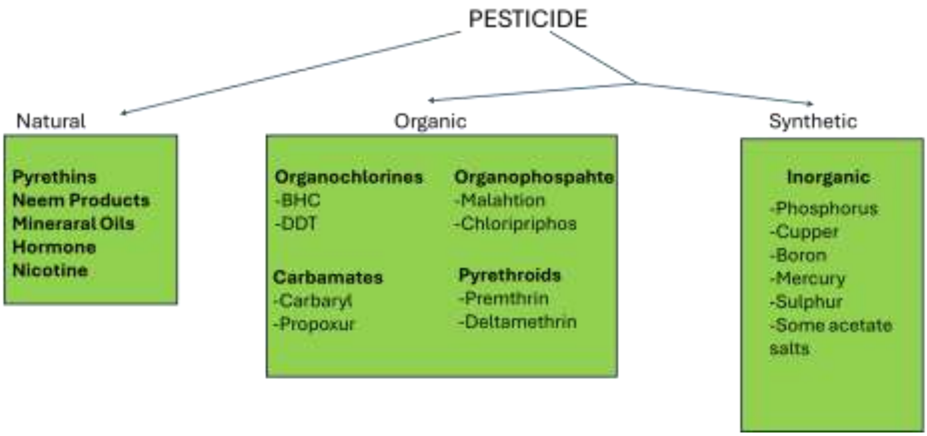

Figure 1. Classification of pesticides

<u>Organic insecticides derived from natural sources:</u>

Green pesticides, usually referred to as environmentally friendly pesticides, consist of a variety of substances that are produced from natural sources. The substances include pheromones, hormones, herbal extracts, and poisons. They include various pest control measures, such as the use of microbial agents, entomopathogenic nematodes, metabolites derived from microbes, and genetically engineered crops that possess resistance against pests and diseases. Biodegradable chemical compounds are being employed more and more in pest management and are acknowledged as pesticides that are benign to the environment.

Synthetic pesticides:

Organophosphates, sometimes referred to as OPs, are chemical compounds formed from phosphoric acid esters. The active ingredient in the pesticide permanently disables the acetylcholine enzyme, which is vital for the proper functioning of the nervous system in insects, animals, and humans. OP rapidly degrades by hydrolysis when exposed to light, air, and soil.

A compound of carbamate, This category comprises organic constituents obtained from carbamic acid (NH_2COOH). The pesticides include a functional compound called carbamate ester. The mechanism of action includes the reversible inhibition of the acetylcholine enzyme.

Organochlorine (OC) is a group of chlorinated compounds that are known for their long-lasting nature. Organochlorine insecticides were once efficacious in controlling malaria and typhus, but they have been banned in almost all industrialized countries. Based on the statistics, 40% of the total pesticides used are categorized as organochlorine. Organochlorine insecticides such as aldrin, hexachlorocyclohexane (HCH), DDT, and dieldrin are widely utilized in economically disadvantaged countries across Asia due to their cost-effectiveness and efficacy against various pests (Abubakar et al., 2019; Nicolopoulou-Stamati et al., 2016; Pathak et al., 2022)

Pyrethroid is a man-made chemical that is created from pyrethrin, a natural substance obtained from chrysanthemum flowersSynthetic pesticides, which are manufactured via chemical processes, are the most often used in modern circumstances (Nicolopoulou-Stamati et al., 2016)

1.2 History of Pesticides

The use of pesticides during history may be classified into three major epochs. Prior to the 1870s, pests were controlled by the use of various natural substances. The Sumerians were pioneers in the use of pesticides, as documented approximately 4500 years ago. Sulfur compounds were used for the purpose of controlling insects and mites. Around 3200 years ago, the Chinese used mercury and arsenical compounds for the purpose of controlling body lice. In the second stage, which occurred between 1870 and 1945, people began using inorganic synthetic materials. During the late 1800s, people in Sweden used copper and sulfur compounds to counteract fungal infections in fruits and potatoes. Later on, people have used other inorganic chemicals, such as the Bordeaux mixture consisting of copper sulfate and lime arsenic, as insecticides to fight against various fungal diseases. These chemicals are still being used for this purpose (Tudi et al., 2021) . The third phase, which started in 1945, included the use of artificial pesticides such as Dichlorodiphenyltrichloroethane (DDT), Hexachlorocyclohexane (BHC), aldrin, dieldrin, endrin chlordane, parathion, captan, and 2,4-D. These pesticides were introduced as a result of their discovered effects. The disadvantages of several of these compounds were their high application rates, lack of selectivity, and significant toxicity(Abubakar et al., 2019; Bertomeu-Sánchez, 2019)

2.Colorimetric methods

Colorimetry is the scientific and technological method used to measure and characterize the physical aspects of human color perception. Colorimetry is traditionally classified into two categories: visual colorimetry and photoelectric colorimetry.

Visual colorimetry is a method that utilizes the human eye to quantify the concentration of a solution by monitoring changes in color. The accuracy of visual colorimetry is lower than that of photoelectric colorimetry due to the difficulty in distinguishing small color variations with the naked eye. Photoelectric colorimetry utilizes advanced instruments such as a photoelectric colorimeter and spectrophotometer.

In other words, Photoelectric colorimetry is a kind of photometry that involves the detection of light and the measurement of changes in its intensity. The term "photo" is derived from the root word that signifies "light." A photometer is an apparatus used to quantify the energy of electromagnetic waves throughout the range of infrared radiation to ultra-violet radiation, which also includes the visible portion of the electromagnetic spectrum

These instruments provide superior accuracy in detecting color changes and determining concentrations. As a result, photoelectric colorimetry finds extensive use across many areas (Fan et al., 2021; Shrestha Kumari & Shrestha, 2024)

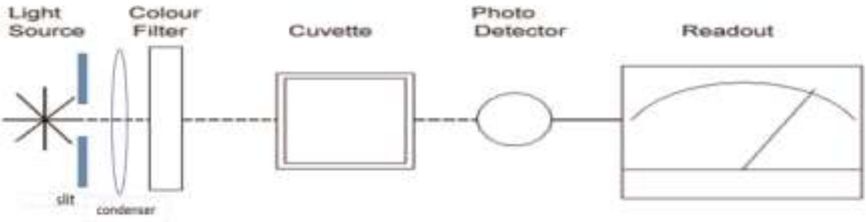

Figure 2. Basic scheme of general colorimetric analyzing device
References.(Shrestha Kumari & Shrestha, 2024)

General basic schema of devices is given at Figure 2. Analyzes can be done quantitatively and qualitatively. Reliable information about the analyte and its concentration can be obtained by measuring the color difference and the change in color tones resulting from the interaction of the analyte and reactants/enzyme/nanoparticle. In this method, UV-VIS adsorption spectrometer is mostly used (Clydesdale & Ahmed, 1978) .

Colorimetry is a technique that quantifies the intensity and wavelength of electromagnetic radiation in the region of vision. It is often used to detect and determine compounds that have the ability to absorb light. It employs two essential principles: Lambert's law, which establishes a relationship between light absorption and distance in an absorbing material, and Beer's law, which establishes a relationship between light absorption and the concentration of a substance. These rules may be consolidated and articulated by equations(Malinin & Yoe, 1961).

2.2 History of Colorimetric Methods

In 1838, W. A. Lampadius conducted one of the first studies on quantitative colorimetric determination. He assessed the quantities of nickel and iron in a cobalt-ore by comparing the color of the filtrate with standard solutions in cylindrical tubes containing varying concentrations of these metals. In subsequent years, similar approaches were used to conduct quantitative investigations on several inorganic substances, including bromine. In 1864, Dehmin presented a novel form of colorimeter for measuring copper levels. This colorimeter, manufactured by Siemens-Halske, was the first commercially accessible instrument for colorimetric analysis (Fernandes et al., 2020).

In 1860, Scottish scientist James Clerk Maxwell created the colorbox, an early kind of colorimeter. This device used a prism to manipulate red, green, and blue light beams individually in order to match the color of a sample. In 1854, Louis Jules Duboscq, a French producer of optical instruments, created the Duboscq colorimeter(Figure 3). In the mid-1930s, photoelectric colorimetry gained widespread recognition. In 1938, William Henry Summerson introduced a colorimeter equipped with a photocell. There is intriguing historical context to the use of photocells, mostly by Germans (Shrestha Kumari & Shrestha, 2024).

Figure 3. Basic scheme of general colorimetric analyzing device
References (Shrestha Kumari & Shrestha, 2024)

The International Commission on Illumination (CIE) established a luminous-efficiency function in 1924 to quantify the significance of light. In 1931, the CIE approved a colorimetry system that included the well-known x, y CIE chromaticity diagram(Figure 4) .The 1931 CIE system of colorimetry was developed using color matching studies carried out independently in England throughout the 1920s by W. David Wright and J. Guild(Boynton, 1996).

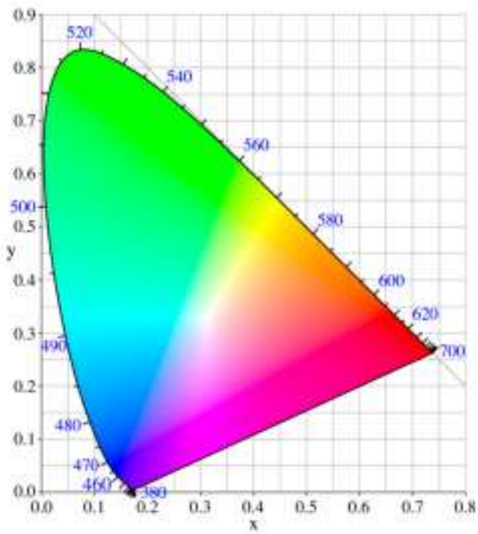

Figure 4. CIE 1931 chromaticity diagram

References (Agudo et al., 2014)

3 Applications

Mane et al. conducted an experiment where they created a mixture of gold nanoparticles and silk fibroin (SF-AuNPs) and used it to detect the presence of the unlabeled organophosphate pesticide chlorpyrifos at a concentration of parts per billion (ppb) using a colorimetric approach they developed.

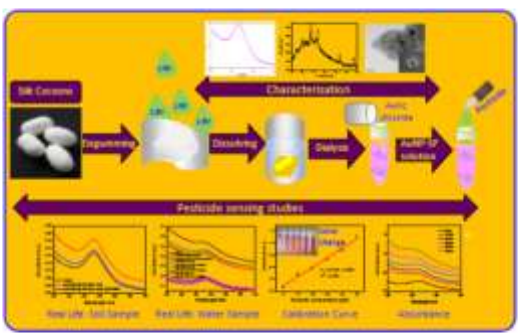

Figure 5. The illustration that shows how silk fibroin-Au bio-nanocomposite dispersion is prepared and used for pesticide sensing
References (Mane et al., 2020)

In this study, MANE et al. They synthesized gold nanoparticles using aqueous fibroin solution obtained from Bombyx mori raw silk. Experimental design is given at Figue 5.They developed a label-free, fast, selective, sensitive and economical colorimetric analysis method of the pesticide chlorpyrifos using silk fibroin-gold nanocomposite material. With the method they developed.They reduced the chlorpyrifos detection concentration by 10 ppb and determined the R2 value as a correlation coefficient of 0.9984 in the calibration they prepared. They also tested the composite structure they prepared on real-life water, soil and vegetable samples and obtained positive and applicable results. By testing other analytes, they showed that the prepared method was selective for the chlorpyrifos analyte. In their report, they stated that the colorimetric method they developed was very economical, easy and sensitive for the primary detection of the presence of harmful pesticides in water reservoirs, soil, fruits and vegetables by the rural population(Mane et al., 2020).

Zhu et al. successfully developed a novel visible microfluidic paper-based analytical device (PAD) in their research. The sensor was fabricated by the use of atom transfer radical polymerization (ATRP). Specifically, pSBMA was immobilized onto the surface of a cellulose filter (CF) and then affixed to the glass surface using PDMS. Three insecticides, namely Chlorpyrifos (CHL), profenofos (PRO), and cypermethrin (CYP), were developed. Simultaneous analysis may be conducted with this sensor. The branch morphology was intentionally crafted to provide many distinct regions for detecting the presence of targeted pesticides. These regions were then treated with particular chemical agents that produce visible color changes upon interaction with the pesticides. Visual perception alone is sufficient to discern variations in color, without the use of any measuring tool. The experimental design is shown in Figure 6

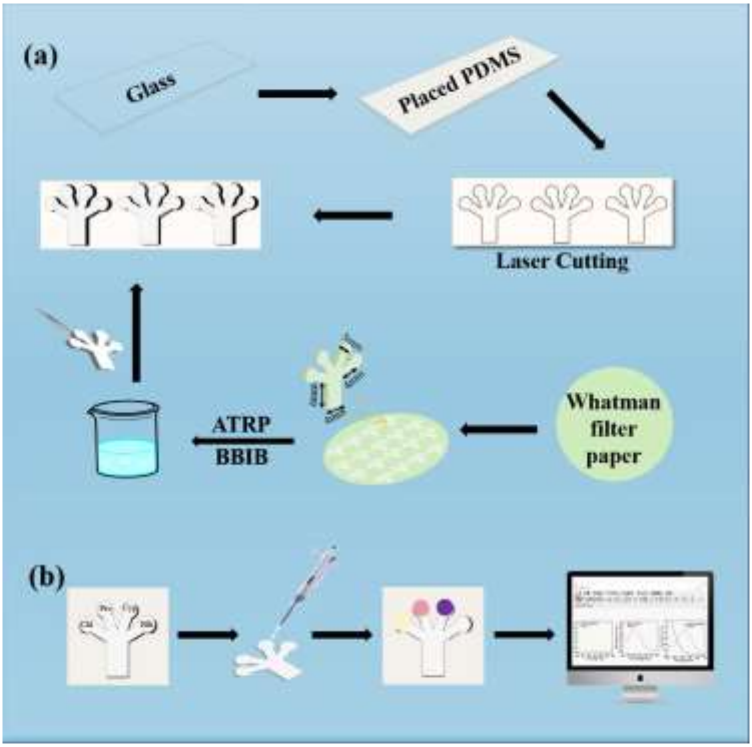

Figure 6. The experimental design paper-based analytical device for colorimetric detection of Chlorpyrifos (CHL), profenofos (PRO), and cypermethrin (CYP). **References** (Zhu et al., 2023)

The colorimetric approach, as it was produced, demonstrated excellent sensitivity, a brief detection time, a strong linear response, and a low detection limit (LOD).The comparison between the data obtained from the proposed paper sensor and those acquired from spectrophotometry demonstrated the stability and reliability of the paper sensor (Zhu et al., 2023).

Wongta et al. aimed to create an in-house colorimetric technique to identify organophosphate and carbamate residues. They used cricket cholinesterase as a cost-effective, user-friendly, and broadly applicable assay. Acetylcholinesterase is an enzyme that hydrolyzes acetylcholine into choline and acetic acid, crucial for pesticide residue detection. It is inhibited by organophosphate and carbamate pesticides. Insects have a faster cholinesterase activity, with most found in the central nervous system. A unique cholinesterase found in crickets' motor end plates is inhibited by physostigmine, making it an attractive alternative for detecting neurotransmitter-inhibiting pesticide contamination (Wongta et al., 2023). Likewise, Wu et al. used the enzymatic reaction approach in the method they developed, but differently, they used gold nanoparticles instead of enzymes. Their approach is described as a colorimetric chemosensor designed to quickly and easily detect the presence of dimethoate pesticide in agricultural goods. The chemosensor primarily depends on the suppression of the peroxidase-like enzymatic activity of gold nanoparticles (AuNPs). The combination of nanoparticles and hydrogen peroxide may cause the substrate o-phenylenediamine (OPD) to undergo oxidation, resulting in the production of the end product 2,3-diaminophenazine. This product exhibits a yellow hue and has a distinctive absorption peak at 450 nm. Dimethoate substantially inhibits the catalytic activity of AuNPs, causing the solutions to become pale yellow or even colorless (Hu et al., 2019).. A further work conducted by Shah et al. used the same approach to produce a reliable and highly sensitive system for detecting organophosphates (OPs). This system utilized the enzyme-like properties of Cysteamine-capped gold nanoparticles (C-AuNPs)(Shah et al., 2021).

A different approach is colorimetric methods developed based on aptamers. There are studies in the literature using this approach for the analysis of pesticide residues. In the study of Tian et al., 37-mer and 25-mer aptamers obtained by cutting from the parental 49-mer were combined with gold nanoparticles and developed a sensitive colorimetric method targeting acetamiprid (pesticide) (Tian et al., 2016). Furthermore, Yang et al. have successfully created a simple and efficient aptasensor for the colorimetric identification of acetamiprid. This was achieved by using composites of hemin functionalized reduced graphene oxide (hemin-rGO). The correlation between the acetamiprid concentration and color fluctuation may be visually assessed and conveniently tracked using a cost-effective UV–vis spectrometer. The aptasensor that was created had a linear response for acetamiprid within the concentration range of 100 nM to 10μM. It was able to detect acetamiprid at a minimum concentration of 40 nM, with a signal-to-noise ratio of 3. This colorimetric aptasensing system has significant benefits, such as a straightforward operational approach, a cost-effective portable equipment, and user-friendly applications (Yang et al., 2014) .

In the developed colometric methods, quantitative measurements and data collection are made using spectrophotometers and photometers. Such devices have been used in the examples given so far in the pesticide analysis focused on in this study. However, developing technology, easier access to information, and the expectation of faster, easier, and more precise analyzes have enabled the development of new approaches. For this purpose, instead of analyzes in the laboratory environment with spectroscopic devices, colorimetric methods have begun to be developed on-site, using portable devices and even smartphones, which are very easy to access, and their applications.

A three-channel colorimetric sensor array has been developed using polydopamine-decorated FeNi foam (PDFeNi foam) to detect antioxidants quickly and effectively. The sensor array can be used with microplate readers and cellphones for signal readouts. PDFeNi foam's catalytic efficiency allows for the rapid oxidation of three common peroxidase substrates in about 3 minutes. The foam's POD-like activity can be modified by π-π stacking and hydrogen bonding, allowing it to discriminate between pesticides on a sensor array. This modular platform offers a straightforward, fast, and efficient method for accurately detecting substances, making it a potential solution for point-of-care diagnostics, food safety, and environmental monitoring(Luan et al., 2024).. Smilarly Wu et al have created a microchemistry analyzer that uses a paper-based colorimetric sensor, Photoshop and a smartphone app to accurately identify carbofuran levels. The approach exhibited exceptional precision with a linear and responsive behavior, as well as low limits of detection. It may also be used for steeping tea. The smartphone application has the capability to attach GPS coordinates to testing sites and send the findings to a website(Wu et al., 2024).

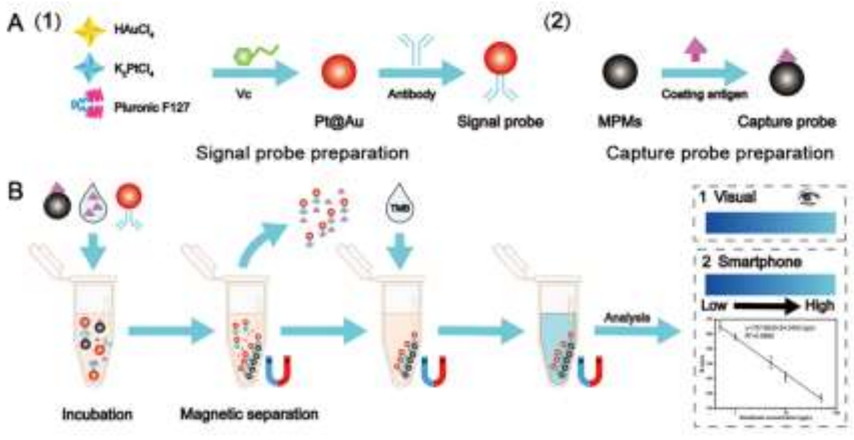

Figure 6. A colorimetric sensor for smartphones for detection omethoate
References (Zhang et al., 2022)

A colorimetric sensor for smartphones has been designed to visually and accurately identify omethoate in fruits and vegetables by Zhang et al... The sensor utilizes an antiomethoate antibody that is coupled to the Pt@Au nanozyme, serving as a catalytic functional signal probe. Additionally, an antigen is attached to magnetic polystyrene microspheres, which act as a separation capture probe. When the separation capture probe is used in conjunction with omethoate, a distinct blue hue becomes apparent, and the smartphone is able to measure the highest B value. Nevertheless, when the quantities of omethoate increase, both the visualization and the B-value acquired from the smartphone decrease. The sensor has the capability to detect omethoate within a linear range of 0.5–50 (µg/L) with LOQ =detection limit of 0.01 (µg/L) (Zhang et al., 2022).

CONCLUSION

The field of analytical chemistry has been at a very critical point in the last fifty years. The reason for this is that the amount of chemicals released into the environment has increased with increasing industry, developing technology and artificial material production, and the analysis of these substances has become inevitable. Although the use of chemical molecules seems necessary for life, it is possible for them to have toxic effects after a certain dose. The example that best explains this situation is pesticides. Pesticides, which are very important for increasing agricultural productivity, cause toxic effects on living things and accumulate in living things after exceeding a certain dose. In fact, the pesticide DDT, which was widely used in history, was banned a few decades later, but its residues were still found even in the poles where agriculture was not practiced due to drift. Pesticide analysis must be very sensitive for both the farmer and the consumer. The farmer's exposure to pesticides during spraying and the accumulation of pesticides in soil and water resources after spraying should be analyzed with high accuracy. At the same time, the fact that the creatures that consume the plant are exposed to the pesticide on the plant, that the pesticide stored in the body causes liver disease, or that there is a risk of genetic mutation for future generations shows how important pesticide analyzes are. For these reasons, different analysis methods have been developed with the developing technology and high sensitivity has been achieved. However, developing knowledge and technology have required these methods to be on-site, fast, cheap and easy. The developed colorimetric methods can be performed cheaply and precisely using spectrophotometer devices. In addition, with the developing technology, on-site colorimetric methods have been developed using smartphone applications. In this way, the farmer or consumer will be able to perform pesticide analysis with their smartphone without needing any expertise. Leaving a sustainable life (a healthy ecosystem without food deficiency) to future generations is one of today's most important goals. These developed methods provide guidance for eliminating the negative effects of pesticide use and taking the necessary precautions.

REFERENCES

Abubakar, Y., Tijjani, H., Egbuna, C., Adetunji, C. O., Kala, S., Kryeziu, T. L., & Patrick-Iwuanyanwu, K. C. (2019). Pesticides, history, and classification. In *Natural Remedies for Pest, Disease and Weed Control* (pp. 29–42). Elsevier. https://doi.org/10.1016/B978-0-12-819304-4.00003-8

Agudo, J. E., Pardo, P. J., Sánchez, H., Pérez, Á. L., & Suero, M. I. (2014). A low-cost real color picker based on arduino. *Sensors (Switzerland), 14*(7), 11943–11956. https://doi.org/10.3390/s140711943

Bertomeu-Sánchez, J. R. (2019). Introduction. Pesticides: Past and Present. *HoST - Journal of History of Science and Technology*, *13*(1), 1–27. https://doi.org/10.2478/host-2019-0001

Boynton, R. M. (1996). Freder,c Ives Medal paper. History and current status of a physiologically based system of photometry and colorimetry. *J.Opt. Soc.Am. A*, *13*, 1609–1621.

Clydesdale, F. M., & Ahmed, E. M. (1978). Colorimetry - methodology and applications. *C R C Critical Reviews in Food Science and Nutrition*, *10*(3), 243–301. https://doi.org/10.1080/10408397809527252

Fan, Y., Li, J., Guo, Y., Xie, L., & Zhang, G. (2021). Digital image colorimetry on smartphone for chemical analysis: A review. *Measurement: Journal of the International Measurement Confederation*, *171*. https://doi.org/10.1016/j.measurement.2020.108829

Fernandes, G. M., Silva, W. R., Barreto, D. N., Lamarca, R. S., Lima Gomes, P. C. F., Flávio da S Petruci, J., & Batista, A. D. (2020). Novel approaches for colorimetric measurements in analytical chemistry – A review. In *Analytica Chimica Acta* (Vol. 1135, pp. 187–203). Elsevier B.V. https://doi.org/10.1016/j.aca.2020.07.030

Hu, Y., Wang, J., & Wu, Y. (2019). A simple and rapid chemosensor for colorimetric detection of dimethoate pesticide based on the peroxidase-mimicking catalytic activity of gold nanoparticles. *Analytical Methods*, *11*(41), 5337–5347. https://doi.org/10.1039/c9ay01506j

Luan, T., Zhang, Y., Song, Z., Zhou, Y., Ma, C. B., Lu, L., & Du, Y. (2024). Accelerated and precise identification of antioxidants and pesticides using a smartphone-based colorimetric sensor array. *Talanta*, *277*. https://doi.org/10.1016/j.talanta.2024.126275

Malinin, D. R., & Yoe, J. H. (1961). Development of the LCIWS of Colotilnetty. *Journal of Chemical Education*, *38*(3), 129–131.

Mane, P. C., Shinde, M. D., Varma, S., Chaudhari, B. P., Fatehmulla, A., Shahabuddin, M., Amalnerkar, D. P., Aldhafiri, A. M., & Chaudhari, R. D. (2020). Highly sensitive label-free bio-interfacial colorimetric sensor based on silk fibroin-gold nanocomposite for facile detection of chlorpyrifos pesticide. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-61130-y

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., & Hens, L. (2016). Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Frontiers in Public Health*, *4*(148).

Pathak, V. M., Verma, V. K., Rawat, B. S., Kaur, B., Babu, N., Sharma, A., Dewali, S., Yadav, M., Kumari, R., Singh, S., Mohapatra, A., Pandey, V., Rana, N., & Cunill, J. M. (2022). Current status of pesticide. *Frontiers in Microbiolgy*.

Shah, M. M., Ren, W., Irudayaraj, J., Sajini, A. A., Ali, M. I., & Ahmad, B. (2021). Colorimetric detection of organophosphate pesticides based on acetylcholinesterase and cysteamine capped gold nanoparticles as nanozyme. *Sensors*, *21*(23). https://doi.org/10.3390/s21238050

Shrestha Kumari, Y., & Shrestha, S. K. (2024). Fundamentals of Colorimetry. In *Advance in Colorimetry*.

Tian, Y., Wang, Y., Sheng, Z., Li, T., & Li, X. (2016). A colorimetric detection method of pesticide acetamiprid by fine-tuning aptamer length. *Analytical Biochemistry*, *513*, 87–92. https://doi.org/10.1016/j.ab.2016.09.004

Tudi, M., Ruan, H. D., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., & Phung, D. T. (2021). Agriculture development, pesticide application and its impact on the environment. In *International Journal of Environmental Research and Public Health* (Vol. 18, Issue 3, pp. 1–24). MDPI AG. https://doi.org/10.3390/ijerph18031112

Wongta, A., Hongsibsong, S., Anand, P., Jaitum, U., Sawarng, N., Wongwilai, W., & Dhumtanom, P. (2023). Developing an in-house colorimetric method for detecting organophosphate and carbamate residue using cricket cholinesterase. *Chemical and Biological Technologies in Agriculture*, *10*(1). https://doi.org/10.1186/s40538-023-00442-3

Wu, K. H., Huang, W. C., Wang, J. C., & Wang, S. H. (2024). Paper-based colorimetric sensor using Photoshop and a smartphone app for the quantitative detection of carbofuran. *Analytical Methods*, *16*(7), 1043–1049. https://doi.org/10.1039/d3ay02211k

Yang, Z., Qian, J., Yang, X., Jiang, D., Du, X., Wang, K., Mao, H., & Wang, K. (2014). A facile label-free colorimetric aptasensor for acetamiprid based on the peroxidase-like activity of hemin-functionalized reduced graphene oxide. *Biosensors and Bioelectronics*, *65*, 39–46. https://doi.org/10.1016/j.bios.2014.10.004

Zhang, B., Zhou, R., Zhang, H., Cai, D., Lin, X., Lang, Y., Qiu, Y., Shentu, X., Ye, Z., & Yu, X. (2022). Smartphone Colorimetric Sensor Based on Pt@Au Nanozyme. *Foods*, *11*(2900).

Zhu, J., Yin, X., Zhang, W., Chen, M., Feng, D., Zhao, Y., & Zhu, Y. (2023). Simultaneous and Sensitive Detection of Three Pesticides Using a Functional Poly(Sulfobetaine Methacrylate)-Coated Paper-Based Colorimetric Sensor. *Biosensors*, *13*(3). https://doi.org/10.3390/bios13030309

CHAPTER 19

MUCUS AND CHLORIDE CELL CHANGES IN GILLS DUE TO SALINITY STRESS

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1. INTRODUCTION

The transition of fish to waters with different salinity levels for various reasons, such as reproduction and nutrition brings about a series of physiological changes. These migrations, mainly observed in diadromous species, are carried out with some metabolic adaptations. Although salinity tolerance in fish varies according to species, there is a broader tolerance range, especially in species living in brackish waters.

It is a natural process for fish to migrate between biotopes with different water characteristics at specific periods of their lives. However, the effects of global warming, the negative consequences of which we have begun to observe more clearly in recent years, require aquatic creatures to adapt to unnatural and changing water properties in their physico-chemical structure. The adverse effects of global warming and climate change directly or indirectly affect natural waters along with all biotopes. The increase in seasonal temperature averages also causes an increase in water temperatures; this causes water to evaporate and withdraw faster than expected, especially in lotic biotopes with closed basin characteristics. The evaporation rate will increase as a natural result of the increase in temperatures. The decrease in the amount of water on a global scale in aquatic environments with salty and brackish water characteristics will increase the amount of salt per unit volume.

Adapting fish species distributed in natural waters to different salinity levels will contribute to successfully implementing fish introduction studies. It will support scientific studies on the protection of fish species against negative scenarios that may be caused by global warming.

This study discusses the physiological responses of individuals and their monitoring in acclimating fish to different salinity levels. Reactions that develop due to increased salinity in fish cause significant changes in the kidneys, especially the gills. However, blood tissue is one of the most essential structures in which physiological response can be measured. The most important of these measurable responses is osmoregulation capacity and blood serum osmolarity increases due to salt increase, thus balancing the fish osmotic pressure.

In the physiological evaluation of salinity tolerance of fish, in addition to serum osmolarity measurement, some tissues and organs of individuals exposed to different salinity levels are dissected, and Na^{+}-K^{+}-ATPase enzyme activities are examined. However, the stress caused by increased salinity can also be measured using Heat shock proteins (HSPs). Proteins frequently used in animal cells for this purpose are HSP60, HSP70 and HSP90. These proteins are synthesized by cells against various biotic and abiotic stress factors (Iwama et al., 1998); these are a family of proteins that play a role in preventing stress-related damage to cells (Feder and Hofmann, 1999). So, monitoring the changes in these proteins effectively reveals the effects of salinity increase.

There are some studies in which the effects of salinity stress are evaluated using different methods. Nordlie et al. (1992) evaluated the adaptation of the *Poecilia latipinna*, whose natural distribution area is fresh and brackish waters, to 70‰ salinity of freshwater individuals and to 0‰ and 80‰ salinity of brackish water individuals. It was determined that 58% of individuals caught in freshwater survived when a salinity level of 70‰ was reached, and 43% of individuals caught in brackish water survived at a salinity of 80‰. While serum osmolarity was measured as 320±2 mOsm/kg at 0‰ salinity level, it was measured as 418±31 mOsm/kg at 70‰ salinity.

Haney (1999) conducted experiments at 7 different salinity levels (2‰, 10 ‰, 20‰, 30‰, 40‰, 50‰ and 60‰) to determine the response of *Cyprinodon variegatus* to salinity increase. While there was no significant difference in serum osmolarity values in the 0-20‰ salinity range, a significant increase was determined after the 20‰ salinity level. Oğuz (2018) studied to reveal the development of the osmoregulatory structures of *Alburnus tarichi* in the larval stage and determined that ionocyte cells were first observed in the larva's yolk sac membrane and then in gills, digestive tract and kidneys. It was reported that the number of these cells determined in the yolk sac and skin decreased due to larval development and that the ionocytes in these tissues played a role in the osmoregulation of the larva in the early life stage. In the salinity stress study conducted by Oğuz et al., 2023 on *Poecilia sphenops* 20‰, 30‰, 40‰ and 50‰ saline in the laboratory were examined. Mucus cells, proliferating cell nuclear antigen, mitochondria-rich cells and heat shock protein (HSP70) cells were marked in the gill tissue in this study. It was determined that the number of mucus cells increased rapidly in the 20‰ salt group and did not change in the 30‰ and 40‰ groups. A rapid increase in mucus cells and HSP70 cell numbers was also observed in the 20‰ and 30‰ salinity groups.

Salinity Acclimation Process

In determining the salinity stress of fish in a laboratory, glass aquariums or tanks of varying sizes and volumes are used depending on the fish species, size and stock density. Two critical questions arise when acclimating fish to different salinities. The first is at what rate the periodic salt increase will be made, and the other is how to determine the maximum salinity tolerance. Determining the maximum salinity tolerance also means terminating the experiment.

Although there are differences in previous studies regarding the amount of periodic salinity increase, it is generally known that a daily salt increase of 2-4 ppt is appropriate. In this regard, Naiman et al. (1976) reported a weekly salinity increase of 17.5‰ (2.5 ppt/day), and Perschbacher et al. (2011) reported that a salinity increase of 4‰ was suitable for acclimatization studies.

There are two similar approaches to trial termination. One of these is that maximum salinity tolerance has been reached with many deaths observed in the groups, and the experiment should be terminated at this stage. Nordlie et al. (1992) determined that approximately 40% of the individuals died, which is a sufficient mortality rate to terminate the experiment. Another approach is that stopping the fish's feed intake is sufficient to stop the experiment.

It is also essential to consider how long exposure of different salinity groups to salinity will be sufficient to produce physiological effects. In this regard, Haney (1999) reported that exposing fish to each salinity level for 5 days is appropriate for determining physiological changes.

Immunohistochemistry (IHC)

After reaching the salinity level determined according to the groups, the dissected gill samples are placed in freshly prepared Bouin's fixative for routine histological processes. After the tissues are kept in fixatives at room temperature for 24 hours, they are transferred to 70% ethanol. It is passed through increasing ethanol concentrations (70%, 80%, 90%, 95% and 100%)

(duplicate, 10 minutes each) and embedded in paraffin blocks. Afterwards, 5 µm sections are taken using a microtome device, and these sections are stained with Periodic Acid-Schiff (PAS), the preparation details of which are given below. Periodic acid is prepared by dissolving 1 g of periodic acid by adding 200 mL of distilled water. While preparing Schiff (PAS), 200 mL of distilled water is boiled, and 1 g of basic fuchsin is added. The resulting solution can cool to 50 °C, and sodium bisulfate is added and mixed. When the solution reaches room temperature, 2 mL HCl and 2 g activated charcoal are added and mixed. The resulting solution is kept in a light-proof bottle at room temperature overnight, then passed through filter paper and stored in the refrigerator at 4 °C. The stained tissues are covered with entellan and examined under a light microscope, and images are taken.

In Hematoxylin-Eosin (HxE) staining, usually, Eosin dye is prepared by dissolving 1 g of eosin oxalate in 100 mL of distilled water. Hematoxylin staining and commercially available dyes are generally used. The resulting sections are taken into chalks, and the process is carried out. The staining process steps of both methods applied to the preparations taken into the chalets are given in Chart 1.

Chart 1. Periodic Acid–Schift (PAS) and Hematoxylin-Eosin (HxE) process steps (Bancroft and Gamble, 2008; Alkan et al., 2023)

	Periodic Acid-Schiff (PAS)		**Hematoxylin - Eosin (HxE)**	
	Transactions	Exposure time	Transactions	Exposure time
1	Xylol-I	5 min.	Xylol-I	5 min.
2	Xylol-II	5 min.	Xylol-II	5 min.
3	100% alcohol	3 min.	100% alcohol	3 min.
4	80% alcohol	3 min.	80% alcohol	3 min.
5	70% alcohol	3 min.	70% alcohol	3 min.
6	Distilled water	5 min.	Distilled water	5 min.
7	Periodic acid	5 min.	Hematoxylin	6 min.
8	Distilled water	Shaking	Distilled water	5 min.
9	Schiff solution	15 min.	Tap water	15 min.
10	Tap water	10 min.	Eosin	3 min.
11	70% alcohol	3 min.	70% alcohol	3 min.
12	80% alcohol	3 min.	80% alcohol	3 min.
13	100% alcohol	3 min.	100% alcohol	3 min.
14	Xylol-I	5 min.	Xylol-I	5 min.
15	Xylol-II	5 min.	Xylol-II	5 min.

At the end of all these processes, the stained tissues are covered with entellan and examined under a light microscope, and images are taken.

There are various cells in the gill tissue, such as mucus, erythrocytes, pavement and chloride (ionocytes, mitochondria-rich cells) cells (Fernandes and Moron, 2020; Alkan et al., 2023). Some histological differences are expected in the gill tissues of fish as a result of exposure to different salinity levels. As a result of Periodic Acid/Schiff staining, changes occur in the mucus cells in the gill tissue. Immunohistochemically marking chloride cells containing Na^+/K^+-ATPase in the gill tissue determines quantitative changes in chloride cells. Cell counting is performed by randomly selecting different regions from tissues marked with Na^+/K^+-ATPase antibodies. Counts are made using a light microscope from randomly selected filament regions in the section (Figure 1).

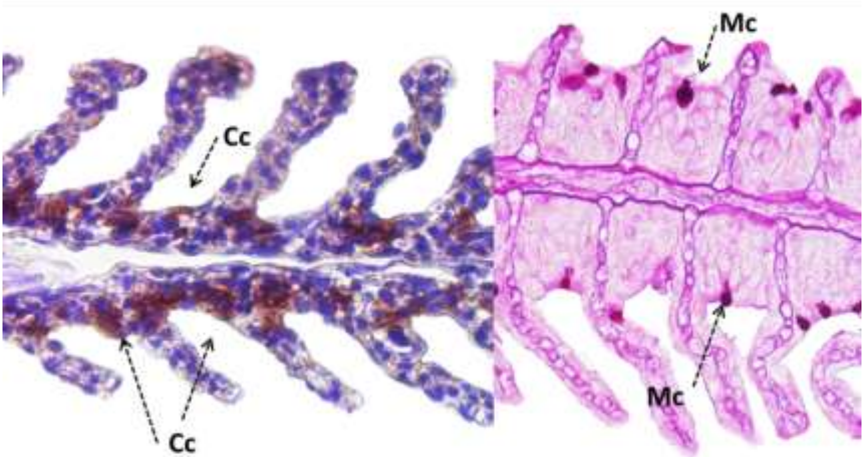

Figure 1. Histological appearance of chloride and mucus cells (Mc: mucus cells, Cc: chloride cells).

2. CONCLUSION

Determining the physiological responses of fish to salinity stress provides data on the salinity tolerance levels of the species. This is crucial for fish species to be stocked or grown in different environments. There are studies in which salinity tolerance is determined by cell changes in blood serum and some related organs (Haney, 1999; Oğuz et al., 2023; Olukolajo et al., 2013).

The most effective method by which the positive or negative effects of changes in the environmental conditions of fish can be observed is histology and blood serum osmolarity studies. Depending on the type of environmental change, the tissues and organs where the mechanism of action occurs vary. Especially when salinity stress is considered, changing ion concentration in water causes significant cellular changes in gill tissues and blood serum osmolarity. Ion balance occurs through various physiological procedures, such as regulating intracellular organic concentration and changes in plasma inorganic ion levels due to osmotic stress (Ballantyne et al., 1987). Organs that are also effective in excretion, such as kidneys, gills, intestines and skin, play a role in creating this ion balance in fish (Demir, 2006). Water forms the fish's external environment; therefore, some of its organs interact directly with water. Due to the gill openings, various chemical changes such as ion exchange, pH, and salinity first affect the gills. Gills are structures with a large surface area concerning the environment and are essential organs to be examined histopathologically in salinity stress studies (Takashima and Hibiya, 1995; Formicki and Kirschbaum, 2019; Oğuz et al., 2023). It has been stated that histopathological damages such as epithelial cell proliferation, epithelial lifting and secondary lamellar fusion, telangiectasis, hypertrophy and necrosis occur in fish exposed to salinity change and that these damages may be caused by a protective response of the gill (Dawood et al., 2022).

Mucus secretion can protect the gills against external factors. The cells that form mucus may differ according to their glycoconjugate content and play a role in protecting the tissue against physical injury (Mittal et al., 1994). It has been reported that there is an increase in the number of mucus and chloride-secreting cells in Silver Carp (*Hypophthalmichthys molitrix*) fish exposed to salinity (Jiang et al., 2022). A similar study stated that the number of Sailfin velifera (*Poecilia velifera*) gill mucus cells increased depending on salinity (Oğuz et al., 2023).

Histopathological studies on related organs and blood tissue studies are decisive in determining the physiological responses of salinity stress in fish when the studies and their results are evaluated.

3. REFERENCES

Alkan, Z., Kaval Oğuz, E., Oğuz, A. R. (2023). Investigation of mucus cell change in gills of van fish (*Alburnus tarichi*, Güldenstädt, 1814) treated with fluvalinate. *Chemistry and Ecology*, *39*(8), 894-907.

Ballantyne, J. S., Moyes, C. D., Moon, T. W. (1987). Compatible and counteracting solutes and the evolution of ion and osmoregulation in fishes. *Canadian journal of zoology*, 65 (8): 1883-1888.

Bancroft, J. D., Gamble, M. (Eds.). (2008). Theory and practice of histological techniques. Elsevier Health Sciences.

Dawood, M. A., Noreldin, A. E., Sewilam, H. (2021). Long-term salinity disrupts hepatic function and intestinal health, and gills antioxidative status in Nile tilapia is stressed with hypoxia. *Ecotoxicol Environ Saf.*, 220:112412. https://doi.org/10.1016/j.ecoenv.2021.112412

Demir, N. (2006). İhtiyoloji. (3. Baskı), Fen ve Biyoloji Yayınları. No: 924, Nobel Basımevi, İstanbul. 223.

Feder, M. E., Hofmann, G. E. (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu Rev Physiol*, 61(1):243–282.

Fernandes, M. N., Moron, S. E. (2020). Breathing and respiratory adaptations. In Biology and physiology of freshwater Neotropical fish (pp. 217-250). Academic Press.

Formicki, K., Kirschbaum, F. (eds) (2019). The histology of fishes. CRC Press. p 456. https://doi.org/10.1201/ 9780429113581

Haney, D. C. (1999). Osmoregulation in the Sheepshead Minnow, *Cyprinodon varigeatus*: influence of a fluctuating salinity regime. *Estuarie*, 22 (4): 1071-1077.

Iwama, G. K., Thomas, P. T., Forsyth, R. B., Vijayan, M. M. (1998). Heat shock protein expression in fish. *Rev Fish Biol Fish*, 8(1):35–56. https://doi.org/10.1023/A:1008812500650

Jiang, Y., Yuan, C., Qi, M., Liu, Q., Hu, Z. (2022). The effect of salinity stress on enzyme activities, histology, and transcriptome of silver carp (*Hypophthalmichthys molitrix*). *Biology*, 11(11), 1580.

Mittal, A. K., Ueda, T., Fujimori, O., Yamada, K. (1994). Histochemical analysis of glycoproteins in the epidermal mucous cells and sacciform cells of an Indian swamp eel *Monopterus cuchia* (Hamilton) (Synbranchiformes, Pisces). *Acta Histochemica et Cytochemica*, 27(3), 193-204.

Naiman, R. J., Gerking, S. D., Stuart, R. E. (1976). Osmoregulation in the Death Valley pupfish *Cyprinodon milleri* (Pisces: Cyprinodontidae). *American Society of Ichthyologists and Herpetologists*, 4: 807-810.

Nordlie, F. G., Haney, D. C., Walsh, S. J. (1992). Comparisons of salinity tolerances and osmotic regulatory capabilities in populations of sailfin molly (*Poecilia latipinna*) from brackish and fresh waters. *Copeia*, 3: 741-746.

Oğuz, A. R. (2018). Development of osmoregulatory tissues in the Lake van fish (*Alburnus tarichi*) during larval development. *Fish physiology and biochemistry*, 44, 227-233.

Oguz, A. R., Sepil, A., Alkan, Z., Ergoz Azizoglu, B., Şen, F., Kaval Oguz, E. (2023). Effects of salinity on gill histology in sailfin velifera (*Poecilia velifera*). *Aquaculture International*, 1-13.

Olukolajo, S. O., Omolara, L. A. A. (2013). Salinity tolerance of grey mullet, *Mugil cephalus* (Linnaeus) fry in the laboratory. *Journal of Fisheries Sciences*, 7 (4): 292-296.

Perschbacher, P. W., Aldrich, D. V., Strawn, K. (2011). Survival and growth of the early stages of Gulf Killifish in various salinities. *The Progressive Fish-Culturist*, 109-111.

Takashima, F., Hibiya, T. (1995). An atlas of fish histology. In: Normal and pathological features, 2nd edn. Kondansha LTD, Tokyo (JP).

CHAPTER 20

GENOME-WIDE ASSOCIATION STUDIES: APPLICATIONS AND ADVANCES IN TREE SPECIES

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1. INTRODUCTION

The purpose of investigating forest tree genomes is to improve our understanding of genetics and develop tools for the conservation and breeding of forest trees. Forest tree research faces several challenges, including as long reproductive cycles, large genomes, limited availability of well-documented mutations for reverse genetic studies, and limited financing. Substantial advancements have been made in the discipline during the past twenty years (Neale & Kremer, 2011). Forest geneticists have improved association genetics as an alternative method for assessing complex features. Genome-wide association studies (GWAS) are currently regarded as an essential approach for examining the genetic composition of traits in tree species. In order to carry out efficient GWAS, a dense set of markers is necessary due to the quick fall of LD. The candidate-gene technique has been commonly utilized for this reason. This difficulty persists until there are readily available reference genome sequences and cost-effective methods for high-density single nucleotide polymorphism (SNP) genotyping or whole-genome resequencing are developed. Currently, it is practically feasible to analyze all single nucleotide polymorphisms (SNPs) present inside genes or across entire genomes. Genome-Wide Association Studies have successfully identified certain genomiec variations of entire genom that are associated with important traits, such as growth rate, disease resistance, and adaptability to variable environments (Table 1). This chapter explores the methodology, significant findings, and future potential of GWAS in tree genetics, with a focus on its use in promoting sustainable forest management and conservation efforts.

Table 1. Complex Traits in GWAS for Tree Species

Family	Genus	Important traits	Important stress
Pinaceae	*Pinus*	Stem growth, Wood density, lignin and cellulose content	Pathogenic Fungi, Cold hardiness, Drought tolerance
	Pseudotsuga	Bud phenology, Wood density	Cold hardiness
Salicaceae	*Populus*	Stem and leaf Growth, Wood density	Pathogenic Fungi, Osmotic potential
Myrtaceae	*Eucalyptus*	Stem growth, Wood density, microfibril angle	Fungal Diseases, Frost tolerance
Fagaceae	*Quercus*	Bud phenology	Drought tolerance
	Castanea	Bud phenology	Drought tolerance

Modified from Neale and Kremer, 2011.

2. METHODOLOGICAL APPROACHES IN TREE GWAS

An excellent tool for identifying potential genes related to various tree traits is the genome-wide association study (GWAS). This method examines the relationship between a molecular marker, such as an SNP, and a target trait. QTL mapping encounters significant constraints when applied to populations with a high degree of organization. The scarcity of recombination events leads to inadequate precision for quantitative features. As Ashwath et al., 2023 stated, the conventional mapping approach is ineffective in perennial plants. Association mapping or GWA stuides overcome these limitations. A GWAS experiment aims to identify marker-trait associations (MTAs) for individual traits and explore the genomic architecture of these traits.

In designing and development of GWAS, it should be selected populations with more genotypes to minimize overestimation of phenotypic variance due to Beavis effects (Ashwath et al., 2023) at first (Figure 1). Secondly phenotyping which is critical for detecting genotype-phenotype connections helps identify unique or combined traits in all genotypes or individuals. Phenotyping can be done at the field level using randomized block designs to sustain effects, or at the nursery level using entirely randomized designs for various type of tree species. Association mapping in plants has utilized co-dominant markers like SSR and SNP genotyping (Zahid et al. 2022). Various high-throughput Genome-wide association studies (GWAS) are a powerful tool for identifying potential genes linked to various tree traits. This method examines the relationship between molecular markers, such as single nucleotide polymorphisms (SNPs), and target traits. Traditional QTL mapping faces significant limitations in highly organized populations due to the scarcity of recombination events, leading to inadequate precision for quantitative traits. As noted by Ashwath et al. (2023), conventional mapping approaches are often ineffective in perennial plants. Association mapping or GWAS overcome these limitations by identifying marker-trait associations (MTAs) and exploring the genomic architecture of traits.

In designing and developing GWAS, selecting populations with a greater number of genotypes is crucial to minimize the overestimation of phenotypic variance due to Beavis effects (Ashwath et al., 2023). Phenotyping is also critical for detecting genotype-phenotype connections, helping to identify unique or combined traits in all genotypes or individuals. This can be performed at the field level using randomized block designs to sustain effects or at the nursery level using completely randomized designs for various tree species.

Association mapping in plants has utilized co-dominant markers such as SSR and SNP genotyping (Zahid et al., 2022). Various high-throughput technologies, including genotyping-by-sequencing (GBS), RAD sequencing, DART sequencing, and chips have been employed for SNP genotyping (Akram et al., 2021). The general linear model (GLM) and mixed linear model (MLM) are commonly used for GWAS analysis (Zhang et al., 2020). GLMs create marker-trait connections by utilizing population structure, phenotype, and genotype data whereas the MLM model uses kinship (K) and population structure (Q). The TASSEL software (Bradbury et al., 2007) is frequently used for GWAS analysis with both SSRs (Lavale et al., 2018) and SNPs. Additionally, PLINK, a tool for whole-genome association, analyzes data from multiple individuals using stratification detection, basic association tests, meta-analyses, gene-based testing, and epistasis screening (Choi et al., 2020).

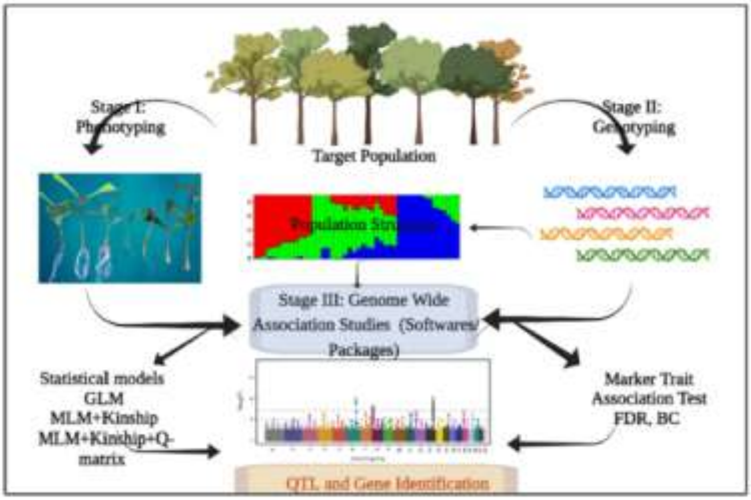

Figure 1. Procedure for GWAS. Reference. Ashwath et al., 2023.

Rare mutations may cause the phenotypic variable of interest, leading to lacking heritability. High-throughput whole-genome sequencing technologies are employed to show the power of GWAS and detect of rare variants (Perea et al., 2016). GWAS software output as result of *Salix* species phenotyping showed the rare but significant SNP (Carlson et al.,2019) hits/positions on related chromosome can be clearly seen in Figure 2.

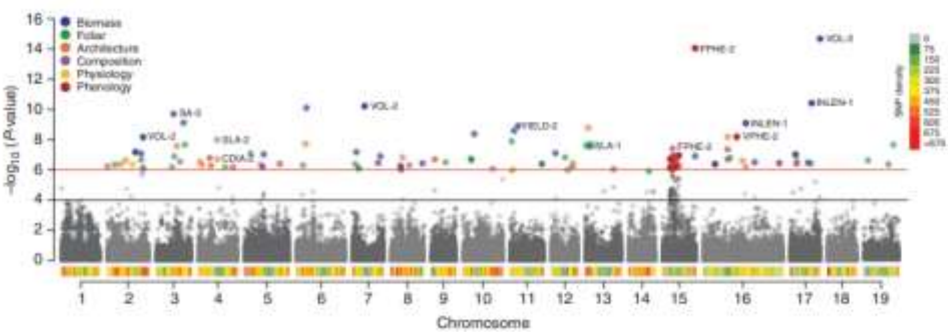

Figure 2. Significant GWAS hits by trait class. Reference. Carlson et al.,2019.

Conventional linkage mapping, or QTL mapping, is often used to identify genomic regions (QTL) that control specific phenotypes. The process occurs during the creation of mapping populations and affects the precision of genetic mapping and the diversity of alleles present (Li et al., 2020). Creating a mapping population using two parent lines that are homozygous for specific traits in tree crops is a difficult and time-consuming task. Ashwath et al. (2023) state that GWAS allows for the development of more detailed maps by taking into account the historical recombination and linkage disequilibrium among representative accessions.

3. CASE STUDIES OF GWAS IN TREE SPECIES

In recent years, several GWA studies have been carried out on important tree species involving *Eucalyptus, Picea, Cryptomeria, Populus, Pinus, and Sequoiadendron.* GWAS has been successfully employed to confirm identified QTLs by interval mapping. Grattapaglia et al. (2011) effectively created the first set of 768 SNPs for the genetically varied *Eucalyptus* genome.

In their study, Eckert et al. (2010) examined a comprehensive dataset of single nucleotide polymorphisms (SNPs) obtained from 1730 specific locations in 682 loblolly pine (*Pinus taeda* L.) trees. These trees were collected from 54 different populations spanning the entire geographic distribution of the species. This study investigated single nucleotide polymorphisms (SNPs) that are linked to geographic location, temperature, growing degree-days, precipitation, and aridity.

In their groundbreaking study, Porth et al. (2013) performed a GWAS on wood chemistry and ultrastructure traits in black cottonwood (*Populus trichocarpa*). They identified 141 significant SNPs that are associated with cell wall qualities. The study was conducted on a sample of 334 unrelated individuals.

Uchiyama et al. (2013) investigated the practicality of performing GWAS in conifers. They used a sample of 367 trees of *Cryptomeria japonica*. Researchers identified six novel Quantitative Trait Loci (QTLs) that have a substantial correlation with differences in wood quality parameters and the quantity of male strobili.

In their study, Zhang et al. (2018) performed GWAS and expression quantitative trait loci (eQTL) analyses on 917 samples of *P. trichocarpa*. They discovered that PtHCT2 plays a role in regulating the levels of chlorogenic acid and other metabolites to a certain degree. This study provides novel insights into the application of omics-based methods for inferring gene function in trees.

Baison et al. (2019) identified 39 novel candidate genes that have an impact on wood formation in *Picea abies* using GWAS. Additionally, they found 52 significant SNPs associated with this trait. The assessed parameters were wood ring density, ring width, width of transitional rings, and the number of cells in the latewood.

In a recent study by Torre et al. (2022), a total of 78 genes that are potentially linked to drought resistance were discovered in *Sequoia sempervirens*, whereas six genes were identified in *Sequoiadendron giganteum.*

4. CHALLENGES IN CONDUCTING GWAS ON TREE SPECIES

Researching trees poses numerous key obstacles for GWA. One primary issue is the long generation times of many tree species, which complicates multigenerational studies and tracking the inheritance of traits over time (Neale and Kremer, 2011). Additionally, many tree species have large and complex genomes, making sequencing and data analysis more challenging and resource-intensive (Mackay et al., 2012). High levels of genetic diversity within and between tree populations further complicate the identification of significant associations between genetic markers and traits (Petit and Hampe, 2006). Trees are also subjected to highly variable environmental conditions, which can influence phenotypic traits and obscure the genetic basis of these traits (Savolainen et al., 2007). Phenotyping challenges add another layer of complexity. Accurately measuring complex traits such as growth rate, wood quality, and disease resistance is labor-intensive, and many traits of interest are polygenic and exhibit phenotypic plasticity (Neale and Kremer, 2011). Additionally, the presence of population structure and relatedness among individuals can lead to false-positive associations, making it crucial yet challenging to properly account for these factors in analysis (Price et al., 2010). Despite increasing numbers of sequenced tree genomes, reference genomes are still lacking for many species, hindering the identification of SNPs and the annotation of genetic variants (Tuskan et al., 2006). Conducting GWAS in trees also requires significant financial and technical resources for sequencing, genotyping, and phenotyping, which may not always be available (Mackay et al., 2012). Integrating GWAS results with other omics data, such as transcriptomics, proteomics, and metabolomics, to understand the functional consequences of identified SNPs can be complex (Evans et al., 2014). Traits of interest in trees often require long-term monitoring, adding logistical challenges and resource demands (Neale and Kremer, 2011). Finally, compared to model organisms, there are fewer genetic tools and resources available for many tree species, limiting the ability to perform functional validation of GWAS findings (Tuskan et al., 2006).

5. FUTURE DIRECTIONS AND EMERGING TRENDS IN TREE GWAS

Future trends in Genome-Wide Association Studies (GWAS) applications are expected to revolutionize our understanding of complex traits in trees by incorporating advanced integrative methodologies. Understanding the genetic foundation and hierarchical relationships of higher-level variations at intermediate levels, such as gene expression, protein composition, and metabolite levels, is crucial for comprehending the complexity of tree traits. Transitioning from genotypic to phenotypic information has proven invaluable for understanding complex quantitative features in trees, as studying "molecular phenotypes" is essential for unraveling the underlying functional processes (Du et al., 2018).

Adding omics-wide data to standard GWAS expands its use and makes it easier to find candidate genes and functional pathways related to target traits (Mizrachi and Myburg, 2016). Various endeavors have connected GWAS findings with RNA-Seq data (Yan et al., 2020), establishing a connection between genes that cause reported phenotypic variations and profiles of cellular transcription activity (Nguyen et al., 2019). The genes adjacent to the GWAS SNPs were analyzed based on their biological function and metabolic environment, showing their possible associations in determining phenotypes related to growth and resistance to abiotic stress (Francisco et al., 2021).

In order to fully reconstruct global biochemical networks using multiple omic layers, it is essential to utilize Omics-Wide Association Study (OWAS) approaches, which depend on the combination of multi-omic measurements and computational data integration. To reliably identify the interacting expression QTLs, it is necessary to use a systematic OWAS technique (Figure 3).

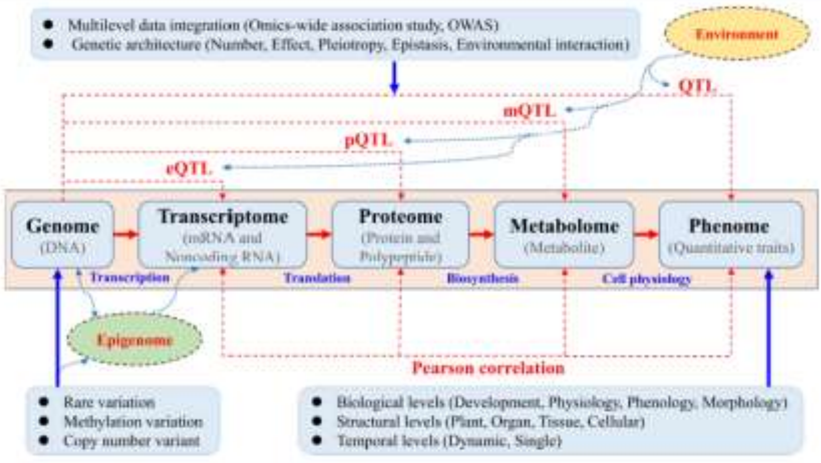

Figure 3. Multiple OWAS mapping of the phenotypes.
Reference. Du et al., 2018.

In conclusion, advancing GWAS in trees requires several key processes. Acquiring reference genome sequences with next-generation sequencing technologies for various tree species is essential. Investing in genetic database resources is necessary, as is the development of improved phenotyping methods (Neale and Kremer, 2011). Employing GWAS tools will facilitate the identification of more causal genes and their functions across multiple phenotypes. This highlights the potential of GWAS to uncover the balanced regulation of these traits and to identify the key hub genes that connect them.

REFERENCES

Akram, S., Arif, M. A. R., & Hameed, A. (2021). A GBS-based GWAS analysis of adaptability and yield traits in bread wheat (Triticum aestivum L.). Journal of Applied Genetics, 62(1), 27–41.

Ashwath, M. N., Lavale, S. A., Santhoshkumar, A. V., et al. (2023). Genome-wide association studies: an intuitive solution for SNP identification and gene mapping in trees. Functional & Integrative Genomics, 23, 297. https://doi.org/10.1007/s10142-023-01224-8

Baison, J., Vidalis, A., Zhou, L., Chen, Z. Q., Li, Z., Sillanpää, M. J., Bernhardsson, C., Scofield, D., Forsberg, N., Grahn, T., & Olsson, L. (2019). Genome-wide association study identified novel candidate loci affecting wood formation in Norway spruce. The Plant Journal, 100(1), 83–100.

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics, 23(19), 2633–2635.

Carlson, C. H., Gouker, F. E., Crowell, C. R., Evans, L., DiFazio, S. P., Smart, C. D., & Smart, L. B. (2019). Joint linkage and association mapping of complex traits in shrub willow (*Salix purpurea* L.). Annals of Botany, 124(4), 701-715. https://doi.org/10.1093/aob/mcz047

Choi, S. W., Mak, T. S. H., & O'Reilly, P. F. (2020). Tutorial: a guide to performing polygenic risk score analyses. Nature Protocols, 15(9), 2759–2772.

Du, Q., Lu, W., Quan, M., Xiao, L., Song, F., Li, P., Zhou, D., Xie, J., Wang, L., & Zhang, D. (2018). Genome-wide association studies to improve wood properties: Challenges and prospects. Frontiers in Plant Science, 9, 1912. https://doi.org/10.3389/fpls.2018.01912

Eckert, A. J., Bower, A. D., González-Martínez, S. C., Wegrzyn, J. L., Coop, G., & Neale, D. B. (2010). Back to nature: Ecological genomics of loblolly pine (*Pinus taeda*, Pinaceae). Molecular Ecology, 19(17), 3789–3805. https://doi.org/10.1111/j.1365-294X.2010.04698.x

Evans, L. M., Slavov, G. T., Rodgers-Melnick, E., Martin, J., Ranjan, P., Muchero, W., & DiFazio, S. P. (2014). Population genomics of *Populus trichocarpa* identifies signatures of selection and adaptive trait associations. Nature Genetics, 46(10), 1089–1096. https://doi.org/10.1038/ng.3075

Francisco, F. R., Aono, A. H., da Silva, C. C., Gonçalves, P. S., Scaloppi Junior, E. J., Le Guen, V., Fritsche-Neto, R., Souza, L. M., & de Souza, A. P. (2021). Unravelling rubber tree growth by integrating GWAS and biological network-based approaches. Frontiers in Plant Science, 12, 768589. https://doi.org/10.3389/fpls.2021.768589

Grattapaglia, D., Silva-Junior, O. B., Kirst, M., de Lima, B. M., Faria, D. A., & Pappas, G. J. Jr. (2011). High-throughput SNP genotyping in the highly heterozygous genome of Eucalyptus: Assay success, polymorphism and transferability across species. BMC Plant Biology, 11(1), 65. https://doi.org/10.1186/1471-2229-11-65

Lavale, S. A., Prashanthi, S. K., & Fathy, K. (2018). Mapping association of molecular markers and sheath blight (Rhizoctonia solani) disease resistance and identification of novel resistance sources and loci in rice. Euphytica, 214(4), 1–11. https://doi.org/10.1007/s10681-018-2156-0

Li, P., Lu, W., Xiao, L., Quan, M., Li, L., Du, Q., & Zhang, D. (2020). Progress and prospect of genome-wide association study (GWAS) in forest trees. Scientia Sinica Vitae, 50(2), 144–153. https://doi.org/10.1360/SSV-2020-0023

Mackay, T. F. C., Stone, E. A., & Ayroles, J. F. (2012). The genetics of quantitative traits: challenges and prospects. Nature Reviews Genetics, 10(8), 565–577. https://doi.org/10.1038/nrg2612

Mizrachi, E., & Myburg, A. A. (2016). Systems genetics of wood formation. Current Opinion in Plant Biology, 30, 94–100. https://doi.org/10.1016/j.pbi.2016.02.007

Neale, D. B., & Kremer, A. (2011). Forest tree genomics: growing resources and applications. Nature Reviews Genetics, 12(2), 111–122. https://doi.org/10.1038/nrg2931

Perea, C., De La Hoz, J. F., Cruz, D. F., Lobaton, J. D., Izquierdo, P., Quintero, J. C., Raatz, B., & Duitama, J. (2016). Bioinformatic analysis of genotype by sequencing (GBS) data with NGSEP. BMC Genomics, 17(5), 539–551. https://doi.org/10.1186/s12864-016-2933-2

Petit, R. J., & Hampe, A. (2006). Some evolutionary consequences of being a tree. Annual Review of Ecology, Evolution, and Systematics, 37, 187-214. https://doi.org/10.1146/annurev.ecolsys.37.091305.110215

Porth, I., Klapšte, J., Skyba, O., Hannemann, J., McKown, A. D., Guy, R. D., et al. (2013). Genome-wide association mapping for wood characteristics in Populus identifies an array of candidate single nucleotide polymorphisms. New Phytologist, 200(3), 710–726. https://doi.org/10.1111/nph.12422

Price, A. L., Zaitlen, N. A., Reich, D., & Patterson, N. (2010). New approaches to population stratification in genome-wide association studies. Nature Reviews Genetics, 11(7), 459–463. https://doi.org/10.1038/nrg2813

Savolainen, O., Pyhäjärvi, T., & Knürr, T. (2007). Gene flow and local adaptation in trees. Annual Review of Ecology, Evolution, and Systematics, 38, 595-619. https://doi.org/10.1146/annurev.ecolsys.38.091206.095646

Tuskan, G. A., DiFazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., ... & Wullschleger, S. D. (2006). The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science, 313(5793), 1596-1604. https://doi.org/10.1126/science.1128691

Uchiyama, K., Iwata, H., Moriguchi, Y., Ujino-Ihara, T., Ueno, S., Taguchi, Y., et al. (2013). Demonstration of genome-wide association studies for identifying markers for wood property and male strobili traits in *Cryptomeria japonica*. PLoS One, 8(11), e79866. https://doi.org/10.1371/journal.pone.0079866

Xiao, Y., Liu, H., Wu, L., Warburton, M., & Yan, J. (2017). Genome-wide association studies in maize: praise and stargaze. Molecular Plant, 10(3), 359–374. https://doi.org/10.1016/j.molp.2016.12.008

Yan, Z., Huang, H., Freebern, E., Santos, D. J., Dai, D., Si, J., et al. (2020). Integrating RNA-Seq with GWAS reveals novel insights into the molecular mechanism underpinning ketosis in cattle. BMC Genomics, 21, 489. https://doi.org/10.1186/s12864-020-06909-z

Zahid, G., Aka Kaçar, Y., Dönmez, D., Küden, A., & Giordani, T. (2022). Perspectives and recent progress of genome-wide association studies (GWAS) in fruits. Molecular Biology Reports, 49(6), 5341–5352. https://doi.org/10.1007/s11033-021-06972-2

Zhang, J., Yang, Y., Zheng, K., Xie, M., Feng, K., Jawdy, S. S., et al. (2018). Genome-wide association studies and expression-based quantitative trait loci analyses reveal roles of HCT2 in caffeoylquinic acid biosynthesis and its regulation by defense-responsive transcription factors in Populus. New Phytologist, 220(2), 502–516. https://doi.org/10.1111/nph.15297

Zhang, Q., Su, Z., Guo, Y., Zhang, S., Jiang, L., & Wu, R. (2020). Genome-wide association studies of callus differentiation for the desert tree, *Populus euphratica*. Tree Physiology, 40(12), 1762–1777. https://doi.org/10.1093/treephys/tpaa115

CHAPTER 21

DETERMINATION OF THE PERFORMANCES OF SOME LOCAL STRAWBERRY VARIETIES İN DİYARBAKIR REGION

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1. INTRODUCTION

Strawberries (Fragaria Although its cultivation is widespread in the world, the countries where it is most produced are China, the United States, Mexico, Spain, Poland and Turkey, respectively. Our country has suitable climate and soil conditions for strawberry cultivation. Strawberry cultivation is done in different regions of Turkey, but the Marmara, Mediterranean, Aegean and Black Sea regions have been the focal points of strawberry production. Strawberries are produced in large quantities, especially in cities such as Antalya, Mersin, Izmir, Bursa and Trabzon. Strawberry cultivation with alternative systems has become increasingly widespread, especially in coastal regions that are known to have a great potential for early strawberry cultivation. Many studies have been carried out in different regions of our country, especially in the Mediterranean, to obtain early crops, extend the production period, protect from adverse climatic conditions, and increase productivity and quality (Özbahçali and Aslantaş, 2015).

Strawberry is one of the fruit species that receives the most breeding work in the world. The aim of these studies is to improve strawberry varieties by making improvements in fruit size, yield, durability, fruit quality and other characteristics. The US states of California and Florida are known as the centers of strawberry breeding studies. These regions are where strawberry cultivation is widespread and this fruit has great commercial importance.

Table 1: Strawberry production areas (ha) by countries between 2017-2021 (FAO, 2023)

	2017	2018	2019	2020	2021
Chinese	107.770	120.476	126.126	126.652	128.537
Russia	24.156	30.063	31.224	32.661	35.466
Poland	49.642	49.180	49.900	33.000	33.900
USA	21.530	1 9.720	17.600	18.818	19.992
Türkiye	15.392	16.102	16.090	17.978	18.676
Egypt	8.085	11.604	14.350	11.850	12.579
Mexican	13.850	13.652	16.429	12.913	11.905
Spain	6.819	7.030	7.260	7.350	7.220
Korea	5.907	6.062	6.462	5.683	6.117
Japan	5.280	5.200	5.110	5.020	4.902
World	370.952	395.178	400.521	383.591	389.665

Strawberry cultivation in the world is carried out with more than 20 species and a large number of varieties in approximately 37 countries in the world, especially due to the unique pleasant smell and taste of strawberry fruit and the nutritional content of strawberries in terms of human health and nutrition (Gaafar and Saker, 2006).

China, which has the largest production area among the strawberry producing countries in the world, produced in an area of 128 537 hectares in 2021, corresponding to approximately one-third of the total strawberry production area in the world. In terms of areas devoted to strawberry production, China was followed by Russia, Poland, the USA and Türkiye, respectively. Due to the interest in strawberries, the areas devoted to strawberry production in the world are increasing day by day (Table.1).

Table 2: Strawberry production values (tons) by countries between 2017-2021 (FAO, 2023)

	2017	2018	2019	2020	2021
Chinese	2.851.100	3.060.300	3.194.409	3.325.833	3.380.478
USA	1.234.130	1.183.870	1.035.100	1.209.730	1.211.090
Türkiye	400.167	440.968	486.705	546.525	669.195
Mexican	658.435	653.639	861.336	557.514	542.890
Egypt	318.950	445.106	545.284	438.730	470.913
Spain	360.416	344.680	351.960	272.550	360.570
Russia	159.900	199.000	208.800	218.400	237.200
Korea	208.699	183.639	234.225	163.646	193.851
Poland	177.921	205.200	185.400	157.600	162.900
Japan	163.700	161.800	165.200	159.200	155.932
World	8.243.750	8.538.477	9.012.638	8.893.590	9.175.384

In terms of world strawberry production amount, China produced approximately more than one third (3,380,478 tons) of the world's 9,175,384 tons of production in 2021. Strawberry production amounts in the world did not follow the table of production areas. China, the leading country in production in the world, was followed by the USA, Türkiye, Mexico and Egypt, respectively. This situation can be explained by the differences in production technologies, techniques, ecologies and varieties of the countries. Our country has an important place in the world in terms of strawberries, like many agricultural products (Table.2).

Table 3: Strawberry production areas in Türkiye between 2019-2023 (TUIK, 2024)

	2019	2020	2021	2022	2023
Mersin	42.192	48.152	44.475	52.329	35.921
Konya	18.073	19.529	20.334	22.903	29.646
Bursa	29.336	30.193	28.239	28.121	28.645
Aydın	16.766	16.879	16.377	24.404	26.549
Çanakkale	6.465	8.364	10.898	16.460	21.410
Antalya	12.160	12.519	14.832	12.172	11.936
Manisa	4.309	8.094	9.655	9.691	9.588
Hatay	1.485	4.140	5.139	7.406	7.240
Elazığ	3.705	3.925	3.975	4.201	4.254
Balıkesir	554	558	2.965	6.574	4.239
Others	25.854	27.424	29.872	38.454	40.407
Total	160.899	179.777	186.761	222.715	219.835

When our provinces are listed in terms of production areas devoted to strawberry cultivation in our country, Mersin comes first. Mersin is followed by Konya, Bursa, Aydın and Çanakkale, respectively (Table. 3).

Table 4: Strawberry production amount (tons) in Türkiye between 2019-2023 (TUIK, 2024)

	2019	2020	2021	2022	2023
Mersin	168.654	188.267	259.958	240.071	167.607
Aydın	67.402	67.698	66.237	95.266	106.272
Çanakkale	23.292	30.098	39.199	62.544	82.504
Konya	43.607	51.098	51.062	60.933	66.908
Bursa	48.465	50.621	48.136	48.093	45.849
Antalya	56.069	51.018	62.488	44.049	40.956
Manisa	14.286	27.451	32.111	32.386	32.033
Hatay	3.658	11.266	16.652	16.333	16.016
İzmir	5.570	5.232	5.281	9.348	13.004
Balıkesir	1.398	1.670	17.323	23.555	11.778
Others	54.304	62.106	70.748	95.534	93.891
Total	486.705	546.525	669.195	728.112	676.818

Although the top 5 provinces of our country that produce the most strawberries are the provinces that allocate the most land for strawberry production, the ranking is not the same. The reasons for this can be considered as ecologies and production techniques. Mersin is again the city with the highest strawberry production in our country. Mersin was followed by Aydın, Çanakkale, Konya and Bursa, respectively. In strawberry production, these five provinces supply more than half of our country's total strawberry production.

Foreign strawberry varieties stand out with their high productivity, ideal flesh hardness, resistance to handling and transportation, and attractiveness. However, these varieties are generally weak in terms of aroma and taste. 'Ottoman', the native and important strawberry variety of our country, is a rare variety with high aroma and taste. However, due to the low productivity and poor attractiveness of the Ottoman variety, interest in this variety has decreased and it is about to disappear. The breeding studies carried out in our country were generally initiated to benefit from the rich aroma and taste of the 'Ottoman' (Paydaş and Kargı, 1992).

Another factor that has contributed to the importance of strawberry cultivation is the benefits that strawberries provide in terms of human health and nutrition. This fruit, which is especially rich in vitamin C, contains up to 100 mg of vitamin C in 100 grams. 100 g of strawberry fruit provides 40-45 calories and contains a significant amount of salicylic acid, vitamins A and B, minerals such as calcium, iron, phosphorus and very small amounts of bromine, silicon, iodine and sulfur. İn addition, strawberries are rich in cellulose, which plays a major role in facilitating digestion. Today, it is also known that strawberries have cancer-preventing properties due to their high ellagic acid content (Ozguven and Yilmaz, 2009).

The fact that most of the varieties used in strawberry cultivation are of foreign origin is an important point showing the need for strawberry breeding studies in Türkiye. Although Türkiye, an important country in world strawberry production, has great potential in strawberry cultivation, it has not made sufficient progress in variety breeding. Strawberry breeding studies in Türkiye started with the aim of combining the prominent aroma and taste characteristics of the local "Ottoman" strawberry variety with more common and productive varieties. While foreign strawberry varieties have advantages such as high yield, durability and transportability, they are generally weak in terms of aroma and taste. On the other hand, the local "Ottoman" strawberry, although rich in taste and aroma, is on the verge of disappearing due to low yield and market appeal. For these reasons, strawberry breeding studies in Türkiye aimed to preserve the aroma and taste of the "Ottoman" strawberry, while developing new varieties by hybridizing with foreign strawberry varieties that have positive characteristics such as higher yield, hardness and durability (Paydas and Kaska, 1992; Ustun and Paydas, 1995).

In the studies carried out in Yalova, 19 types were selected as candidate varieties from the crossbreeding of 'Osmanli', 'Yalova 104', 'Tufts', 'Cruz' and 'Tioga' in order to obtain varieties with high fruit flesh hardness that are suitable for table use and deep freezing. (Erenoglu et al., 1998). İn the studies carried out as a continuation of this study, among the previously selected types; 7 promising types with the codes 92.15.1, 92.1.1, 92.71.2, 92.86.6, 92.18.5, 92.77.2 and 92.100.9 were named 'Doruk 77', 'Dorukhan 77', 'Hilal 77' in 2012, respectively. İt was registered under the names 'Ata 77', 'Bolverim 77', 'Eren 77' and 'Erenoglu 77' (Erenoglu and Seniz, 2002).

This study, which examined the yield, development and fruit quality of 7 hybrid varieties bred and registered in Yalova in different climate and soil conditions such as Diyarbakır, was carried out in order to help Turkey diversify strawberry production and make it sustainable in more regions.

2. MATERIALS AND METHODS

2.1. Material

The research was conducted between 2016 and 2018 on the land of Diyarbakır GAP International Agricultural Research and Training Center. In the project, adaptation trials of 7 strawberry varieties (Erenoğlu 77, Dorukhan 77, Doruk 77, Bolverim 77, Hilal 77, Eren 77, Ata 77), which were bred and registered by the Atatürk Horticulture Central Research Institute, were carried out in Diyarbakır conditions.

When the meteorological data received was examined, the highest value among the maximum temperatures in 2016 was determined as 42.6 °C in July, and the minimum lowest temperature was determined as -19 °C in January. When the meteorological data in 2017 was examined, the highest maximum temperature was 44 °C in August 2017, and the lowest minimum temperature was -12.1 °C in February 2017. When the meteorological data of 2018 was examined, it was seen that the highest maximum temperature was 44.2 °C in July and the lowest minimum temperature was -4.9 °C in December.

Picture 1: Strawberry trial plot image in 2017

Erenoglu 77: It is a hybrid of Cruz x Tioga varieties. It's large and bright red fruits have a low resistance to breaking from the stem and are heart-shaped. In addition to its high table quality, it is also a highly productive variety that is suitable for deep freezing.

Dorukhan 77: It is a hybrid of Tufts x Cruz varieties. Its medium-sized and bright red fruits have a hard flesh and low resistance to breaking from the stem. In addition to its high table quality, it is also a highly productive variety that is suitable for deep freezing.

Doruk 77: It is a hybrid of Tufts x Cruz varieties. Its bright red colored fruits are small and their flesh is hard. The resistance of fruits to breaking off the stem is low. It is a variety suitable for deep freezing and can also be used for table use.

Bolverim 77: It is a hybrid of Tioga x Yalova-104 varieties. The fruits are light red, shiny on the outside and large. It is a variety with flat-cornered fruit shapes, medium quality taste, and suitable for deep freezing.

Picture 2: Strawberry trial plot image in 2018

Hilal 77: It is a hybrid of Ottoman x Tufts varieties. Its high-quality, bright red fruits are heart-shaped and medium sized. The fruit flesh is hard, its smell and taste are quite good. The resistance of the fruits to break off from the stem is low. It is a table variety and is also suitable for deep freezing.

Eren 77: It is a hybrid of Ottoman x Tufts varieties. The fruits are bright red, conical in shape and medium sized. The fruit is of high quality, its flesh is hard, and its taste and smell are quite good. It is a table variety and is also suitable for deep freezing.

Ata 77: It is a hybrid of Tioga x Cruz varieties. Its high-quality, bright red fruits are medium-sized and heart-shaped. The fruit flesh has a firm taste and smell is quite good. The resistance of the fruits to break off from the stem is low. It is a table variety and is also suitable for deep freezing.

Picture 3: A view of the Dorukhan 77 variety

2.2. Methods

The experiment was set up with 4 replications according to the randomized block design and 30 plants were planted in each replication. İn the experiment where fresh seedlings were used, the plants were planted on the prepared banks of 65–70 cm in width and 20–25 cm in height, with a distance of 30 cm between the rows and 25 cm between the rows, using the cross planting method on 12/11/2015. The embankments were mulched with black plastic. İn 2016, the first year of the trial, sufficient yield could not be obtained from the varieties, but yields could be obtained in 2017 and 2018.

The following phenological observations, measurements and analyzes regarding earliness, yield, quality and plant growth were made in the experiment: The first flowering date is the date when the first flowers are seen. First and last harvest dates are the dates when the first and last harvests were made. Harvest time is the time between the first and last harvest date. Yield per plant (g/plant) was calculated by weighing the yield amount in each parcel at each harvest on a scale sensitive to 0.1 g and dividing the total amount of product obtained from each parcel by the number of plants in the parcel. Fruit weight (g) was obtained by dividing the yield obtained from each plot by the number of fruits at each harvest. Evaluations were made sensory by fruit firmness and fruit flesh hardness. Sensory evaluation was scored by a team of

5 people with a score of 1–5. Water soluble total dry matter contents (TSDM) (%) were determined by reading with a hand refractometer. Titratable acid content (%) was determined in terms of citric acid by the titratable acidity method.

Picture 4: A view from the trial area

Statistical Analysis

In the study, pomological and chemical analyzes were carried out on 20 fruits from each variety and the obtained data were processed into the Microsoft Excel program. Statistical analyzes were performed using the SPSS package program (SPSS, 2012). More than two subgroups of the factors deemed important in the analysis of variance were compared with the Duncan test.

3. FINDINGS AND DISCUSSION

Flowering and harvest dates and harvest times of the strawberry varieties examined in the experiment are given in Table 1. Since fruit setting did not occur in 2016, which was the first year of the study, it was not taken into consideration, and 2017 was accepted as the first year since it was the first year in which data was obtained. İn 2018, flowering occurred approximately one week earlier than in the previous year. The first variety to bloom on March 30 in 2017 and February 24 in 2018 was the ATA 77 variety in both years of the experiment (Table. 5, Table. 6, Table.7).

Table 5: 2016 Climate data (General Directorate of Meteorology)

	Months (2016)											
	January	February	March	April	May	June	July	August	September	October	November	December
Maximum Temperature (°C)	11,2	21,8	21,1	28,8	32,9	40,5	42,6	42,2	37	31,5	22	11,4
Minimum Temperature (°C)	-19	-5,6	-5,1	-0,3	5,2	11,6	17,2	18,6	7,7	6,1	-5,8	-9,8
Average Temperature (°C)	1,1	7,9	9,7	15,7	19,9	26,8	31,6	31,9	24,2	18,8	8,2	2,4
Average Humidity (%)	79,3	71,7	66,1	56,2	51,9	32	23	22,7	29,9	36,9	54	74,6

The first varieties harvested in 2017 were Doruk 77, Eren 77 and Erenoglu 77 (6 May), while the latest harvested variety was Bolverim 77 on 15 May. İn 2018, the first variety harvested was Eren 77 (May 3) and the last variety was Bolverim 77 (May 11). The last harvest in 2017 was on June 16 and in 2018 on June 12. When the varieties are compared in terms of the time between the first harvest and the last harvest; The longest harvest period was in Eren and Erenoglu varieties in 2017; İn 2018, it was determined in the Erenoglu 77 variety, while the shortest harvest period was observed in the Ata 77 variety in both years (Table. 5, Table. 6, Table.7).

Table 6: 2017 Climate data (General Directorate of Meteorology)

	Months (2017)											
	January	February	March	April	May	June	July	August	September	October	November	December
Maximum Temperature (°C)	12,7	18	20,5	25,6	33	41,8	43,5	44	40,4	29,4	21,5	16,8
Minimum Temperature (°C)	-10,3	-12,1	-0,5	0,3	6,8	11,6	17,2	17,1	12,8	5,7	-1,4	-4,9
Average Temperature (°C)	1,5	1,5	9,4	12,8	18,8	26,9	32,3	31,2	26,8	17,2	10	5,8
Average Humidity (%)	71,6	62,3	69,5	68,5	57,6	30	19,4	22,8	22,3	39,2	67,5	74,1

The harvest periods of the varieties used in the study were shorter compared to other studies using the same varieties (Demirsoy et al., 2017). This situation is directly related to the ecological situation of the region where the study was conducted and the compatibility of strawberry varieties.

Table 7: 2018 Climate data (General Directorate of Meteorology)

	Months (2018)								
	January	February	March	April	May	June	July	August	September
Maximum Temperature (°C)	14,9	16,8	24,5	29	33,3	41	44,2	41	39,5
Minimum Temperature (°C)	-4,2	-3,1	1,1	2,9	4,6	7	17,2	17,9	13,3
Average Temperature (°C)	5,2	7,6	12,4	15,9	19,4	26,6	31,2	31,4	26,1
Average Humidity (%)	76,9	74,5	62,8	52,9	67,3	37,4	24,1	24,1	29,3

The yield and some fruit quality characteristics of the strawberry varieties included in the trial are given in Table 2. When the data taken for two years was examined statistically, it was seen that the yield per plant was important. The highest yields per plant were obtained from Ata 77 and Hilal 77 varieties, and the lowest yields were obtained from Doruk 77 varieties. Additionally, more productivity was achieved in 2018 than in the previous year. Although the varieties display different characters in terms of yield per plant compared to another study using the same varieties (Demirsoy et al., 2017), it is seen that the yield per plant is similar to the yields in other studies and is even quite high considering the shortness of the harvest period (Özbahçeli and Arslantaş, 2015). ; Soysal et al., 2019; Gündüz and Gökçek, 2019).

Strawberries are usually sold to the consumer by the pound or by the package. Therefore, fruit weight directly affects commercial value. Differences between varieties in terms of fruit weight were found to be statistically significant in both years of the study. The highest fruit size was recorded in the Bolverim 77 (11.52 g) variety and the lowest in the Eren (7.45 g) variety, and these values were similar to the values shown in other locations of the varieties (Demirsoy et al., 2017).

Table 8: Flowering, harvest dates and harvest duration of the strawberry varieties included in the trial

Varieties	First flowering		First harvest		Last harvest		Harvest Season (Days)	
	2017	2018	2017	2018	2017	2018	2017	2018
ATA 77	30.3	24.2	8.5	7.5	1.6	27.5	23	21
BOLVERİM 77	3.4	26.3	15.5	11.5	14.6	11.6	30	31
DORUK 77	6.4	27.3	6.5	6.5	12.6	7.6	37	32
DORUKHAN 77	5.4	27.3	7.5	4.5	15.6	8.6	39	35
EREN 77	2.4	26.3	6.5	3.5	15.6	5.6	40	33
ERENOGLU 77	31.3	24.3	6.5	4.5	15.6	11.6	40	38
HİLAL 77	1.4	25.3	8.5	6.5	16.6	12.6	39	37

The SSC content, which reflects the taste profiles of strawberry fruits, is affected by various factors such as climatic conditions, years, varieties and yield per plant. The differences between the SSC contents of the varieties were found to be statistically significant in both years of the experiment. While the Hilal 77 variety had the highest value in terms of SSCM content in the average of the two years, with 8.51%, the lowest SSCM content was found in the Eren variety (7.55%), and Doruk 77 variety (7.59%) was statistically in the last group. In addition, it was observed that the SÇKM rates in 2018 were lower than in 2017, and it was concluded that this situation was related to the harvest period and yield. However, it was clearly revealed that the SSCM data obtained in the study was similar or higher compared to other studies (Soysal et al., 2019; İslam et al. 2019; Özbahçeli and Arslantaş, 2015; Özgüven and Yılmaz, 2009).

Picture 5: A view from harvest time in the trial field

Another important component that determines taste is the acid content of the fruits. The acid amounts measured in the fruit juices of the varieties included in the study during the harvest period are given in Table 2. As a result of the statistical analysis, it was seen that the acid content of the fruits is important. Among the varieties, the highest acid content was measured in Bolverim 77 variety, and the lowest acid content was measured in Erenoğlu 77 variety. No significant difference was found in acidity values between the years the study was conducted. Özuygur (2005) reported that the titratable acidity rate was 0.92% in the 'Osmanlı' variety and 0.75% in the 'Sweet Charlie' variety; Çelebioğlu (2015) reported the titratable acid rate as 1.08% in the 'Osmanlı' variety, 0.82% in the 'Sweet Charlie' variety, and 1.26% in the 'Hairy' variety. It is known that the acid contents of the varieties in this study are higher compared to some other studies (Soysal et al., 2019; Sarıdaş et al., 2019; Attar and Kargı, 2019) and that this is affected by many factors.

One of the most important quality parameters of strawberries that affects consumer preferences and post-harvest durability is fruit hardness.

Fruit firmness is one of the most important quality parameters that determine the post-harvest strength of strawberries and consumer preference (Kaşka et al., 1986). In the sensory analysis carried out by a team of 5 people, it was recorded that Doruk 77 and Dorukhan 77 varieties had the hardest fruits, and Eren variety had the lowest values. Fruit firmness is a feature that can be affected by various factors, and some of these factors are variety characteristics, the ecology in which it is grown, planting times (spring, summer, autumn, winter), growing systems (open and under cover), planting systems (tube, refrigerated seedling, vertical bag). culture), mulch applications, plant growth regulators (hormones, bacterial applications) and fertilizer applications. High temperatures are also an important factor that reduces fruit flesh firmness (Alan 2013, Agüero et al., 2015, Kandemir 2016, Mısır 2016). It was concluded that the fruit flesh firmness of the varieties examined in the study was sufficient.

Table 9: Yield and some fruit quality characteristics of the strawberry cultivar in the experiment

Varieties	Yield per plant (g)	fruit weight (g)	SSC (%)	TA (%)	Fruit firmness (Sensory: 1-5)
ATA 77	432.67 a	8.72 c	8.05 b	0.73 c	3.15 b
BOLVERİM 77	278.48 b	11.52 a	8.15 b	0.90 a	3.18 b
DORUK 77	174.72 c	8.50 cd	7.59 c	0.68 e	3.36 a
DORUKHAN 77	307.42 b	10.07 b	7.96 b	0.87 b	3.35 a
EREN 77	295.86 b	7.45 e	7.55 c	0.71 d	3.02 c
ERENOGLU 77	283.84 b	9.96 b	7.93 b	0.64 f	3.22 b
HİLAL 77	386.98 a	7.84 de	8.51 a	0.74 c	3.20 b
Importance level	0.000	0.000	0.000	0.000	0.000
Yillar					
2017	274.2 b	8.84 b	8.24 a	0.75	3.15 b
2018	342.93 a	9.46 a	7.69 b	0.75	3.27 a
Importance level	0.000	0.000	0.000	0.799	0.000
Overall	308.57	Eyl.15	Tem.96	0.75	Mar.21

*: The difference between means with the same letter in the same column is significant at the p<0.05 level.

4. CONCLUSION

In the study, Ata 77 variety stood out in terms of productivity. However, the fruits of this variety remained small and showed average values in other parameters. Therefore, Ata 77 variety can be recommended for use in jam and industry. Hilal 77, the other variety in the first group in terms of yield, stood out with its high SSC content and pleasant aroma. Although there were no outstanding varieties in all examined parameters, Dorukhan 77 variety showed average values in terms of yield, but showed above average values in terms of fruit flesh hardness and fruit size and was recommended for table consumption.

REFERENCES

Aguero, J.J., Salazar, S.M., Kirschbaum, D.S. & Jerez, E.F. (2015). Factors affecting fruit quality in strawberries grown in a subtropical environment. İnternational Journal of Fruit Science, 15: 223-234.

Ağaoğlu, Y.S. (1986). Grape-like Fruits. Ankara University Faculty of Agriculture Publications: 984. Textbook: 290. p377.

Alan, F. (2013). Research on the determination of the performance of some day-neutral strawberry (Fragaria x ananassa Duch.) varieties under Kayseri conditions. Master's Thesis, Ataturk University, İnstitute of Natural and Applied Sciences, Department of Horticulture, Erzurum.

Attar, S.H. & Paydas Kargi, S. (2019). Determination of Some Fruit Quality Parameters of Selected Superior Hybrid Strawberry Genotypes and Their Parents. Bahce 48 (Special İssue 1: 6th National Berries Symposium): 111–119 (2019) İSSN 1300–8943.

Celebioglu, B. (2015). The effect of some pollinator strawberry varieties on the fruit characteristics of Ottoman strawberries. Master's Thesis, Gaziosmanpasa University, İnstitute of Natural and Applied Sciences, Department of Horticulture, Tokat.

Demirsoy, L., Kose, D. & Demirsoy, H. (2017). Yield, Quality and Plant Growth Characteristics of Some New Strawberry Varieties Bred in Our Country. Bahce, 46(special issue 1), 97 - 106.

Erenoglu, B., Erbil, Y. & Ufuk, S. (1998). Strawberry Breeding by Crossbreeding–1: Ataturk Horticulture Central Research İnstitute Publications, Scientific Research and Reviews Publication No: 100. 56s.

Erenoglu, B. & Seniz, V. (2002). İnvestigations on Differences in Fruit Yield and Quality of New Strawberry Hybrid Cultivar Candidates Obtained From a Cross Breeding Program. Acta Horticulturae 567:169–172.

Gaafar, R.M. & Saker, M.M. (2006). Monitoring of cultivars identity and genetic stability in strawberry varieties grown in Egypt. World J. Agric. Sci. 2 (1). 29–36.

Gunduz, K. & Gokcek O. (2019). Determination of Yield and Fruit Quality Characteristics of Strawberries Grown in Glass Greenhouses in Soilless and Soiled Environments. Garden, 48(1), 101 - 110.

Hancock, J. F. (1999). Strawberries. Printed&Bound in the UK at University Press, Cambridge P 231.

İslam, A., Bankaoglu, İ. & Turan, A. (2019). Determination of yield and quality characteristics of some strawberry varieties grown in high tunnel in Camoluk district of Giresun province. Yalova Ataturk Horticulture Central Research İnstitute Journal, Bahce 48 (Special İssue 1: 6th National Grape Fruits Symposium): 177-185.

Kandemir, A. (2016). Determination of the performance of some strawberry varieties under greenhouse conditions. Master's Thesis, Ondokuz Mayis University, İnstitute of Natural and Applied Sciences, Department of Horticulture, Samsun.

Kaska, N., Yildiz, A., Paydas, S., Bicici, M., Turemis, N. & Kuden, A. (1986). Effects of greenhouse cultivation of some new strawberry varieties for Turkey with summer and winter planting systems in Adana on yield, quality and earliness. Natural Science Journal D, 2: 10.

Misir, D. (2016). Adaptation of some strawberry varieties. Master's Thesis, Ondokuz Mayis University, İnstitute of Natural and Applied Sciences, Department of Horticulture, Samsun.

Ozbahçali, G. & Aslantaş, R. (2016). Determination of the Performance of Some Strawberry Varieties (Fragaria X ananasss Duch.) in Erzurum Ecology. Atatürk University Faculty of Agriculture Journal, 46(2), 75-84.

Ozgüven, A.I. & Yılmaz. C. (2009). Morphological and Pomological Characteristics of Some Strawberry Varieties in Adana Ecological Conditions. Alatarim 2009. 8 (2): 17-21.

Ozuygur, M. (2005). Determination of Yield, Fruit Quality Criteria and Plant Characteristics of Some Local, American and European Origin Strawberry Varieties and Some Hybrid Strawberry Genotypes in Adana Conditions. Master's Thesis. Cukurova University İnstitute of Science and Technology, Adana, 151 p.

Paydas, S. & Kaska, N. (1992). Performances of Some Promising Strawberry Varieties Obtained by Crossbreeding Breeding in Adana and Pozanti Ecological Conditions. Turkiye 1st National Horticulture Congress Vol.1 p:535–539.

Soysal, D., Demirsoy, L. & Demirsoy, H. (2019). Yield and Quality Characteristics of Some Strawberry Varieties in Samsun Ecology. Garden, 48(1), 45 - 50.

Saridas, M.A., Bircan, M., Karasahin, Z., Kafkas, E. & Paydas Kargi, S. (2019). Change of Some Pomological Characteristics in Selected Strawberry Genotypes with Crossbreeding Breeding During the Active Harvest Season. Yuzuncu Yil University Journal of Agricultural Sciences Volume 29, İssue 3.

Ustun, P. & Paydas, S. (1995). Research on Yield and Fruit Quality of Some Hybrid Strawberry Variety Candidates. Turkiye II. National Horticulture Congress Vol.1 p:301–305.

CHAPTER 22

A GENERAL OVERVIEW OF OLIVE CULTIVATION IN TURKIYE AND IN THE WORLD

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1. INTRODUCTION

Domestication, based on natural selection in species made consciously by humans over many years, generally aims to meet human requirements (Zedar et al., 2006; Marie et al., 2012). But there are difficulties in unraveling the history of domestication. One of the oldest indicators and species of Mediterranean civilizations is the olive tree (*Olea europaea* ssp. *europaea*) (Besnard et al., 2013). It is not known exactly where the olive was first domesticated. However, since humans domesticated the first plants and animals in the Fertile Crescent, it is conceivable that olives also began to be domesticated near here (Lanza, 2012). Olive horticulture began approximately 6000 years ago in this region and spread from that region to Palestine, Israel, Türkiye, Lower Egypt, the Aegean islands, the lands of Greece, the coastal regions of the Balkans, Spain and Italy (Fig. 1) (Cimato and Attilio, 2011). Evidence found in the regions of Syria, Palestine and Crete is the oldest evidence of the cultivation and commercialization of olives.

Figure 1: Distribution of cultivated (*Olea europaea* subsp. *europaea*) and wild olives (*Olea europaea* subsp. *oleaster*) over time in the Mediterranean basin (Carrión et al., 2010; Langgut et al., 2019; Langgut and Garfinkel 2022)

2. TAXONOMY OF THE OLIVE TREE

Olive is one of the main species of the *Oleaceae* family, which includes 30 genera and 600 species (Cronquist, 1981; Chiappetta and Muzzalupo, 2012), and the Olea genus includes 30 species (Bracci et al., 2011) and 6 defined subspecies (Table 1) (Green, 2002). These species within the Olea genus are distributed in Europe, Asia, Oceania and Africa (Bracci et al., 2011; Chiappetta and Muzzalupo, 2012).

The genus *Olea* is divided into 3 subgenera; *Tetrapilus*, *Paniculatae* and *Olea*. *Olea* subgenera includes cultivated and wild olives (Table 1) (Rugini et al., 2011; Chiappetta and Muzzalupo, 2012). Cultivated olives (*O. europaea* L. subsp. *sativa* or *O. europaea* L. subsp. *europaea)* and wild olives *(O. europaea* L. subsp. *oleaster* or O. *europaea* L. subsp. *sylvestris)* (Bellini et al., 2008; Fanelli et al., 2022) are diploid species (2n = 2x = 46) (Chiappetta and Muzzalupo, 2012; Rugini et al., 2011). It has been emphasized in many studies that *O. europaea* subsp. *europaea* var. *sylvestris* is the wild relatives of cultivated olives (Besnard and Rubio de Casas, 2016; Falek et al., 2022).

Table 1. Taxonomic classification of *Olea europaea* L. (Rugini et al., 2011; Chiappetta and Muzzalupo, 2012).

Kingdom:	*Plantae*
Phylum:	*Magnoliophyta*
Class:	*Rosopsida*
Order:	Lamiales
Family:	*Oleaceae*
Sub-family:	*Oleideae*
Genus:	*Olea*
Sub-genera:	*Paniculatae* *Tetrapilus* *Olea*
Sections:	*Ligustroides*
***Olea* Sub-species:**	*cuspidata* *laperrinei* *maroccana* *cerasiformis* *guanchica* *europaea* **cultivars***:* *sylvestris* (wild olive) *europaea* (cultivated olive)

3. WORLD OLIVE PRODUCTION

Olive culture dates back to ancient times. Therefore, olive has played a very important role in human lives and also pioneered the development of culture and food (Cimato and Attilio, 2011). Olives are one of the most important element of the Mediterranean diet. The demand for olive products has increased tremendously due to increased awareness about the gastronomic and health benefits of table olives and olive oil (Erel et al., 2013, Nteve et al., 2024). As a result, this contributed to the development of the olive industry.

Approximately 95% of olive production areas are located in the Mediterranean Region. The most important olive producing countries include Spain, Italy, Greece, Türkiye, Tunisia, Syria, Morocco, Portugal, France and Algeria. In the last decade, the world average olive production has been of 21,227,000 tons, while olive oil has been of 3,123,000 tons (Fig. 2) (FAO, 2024).

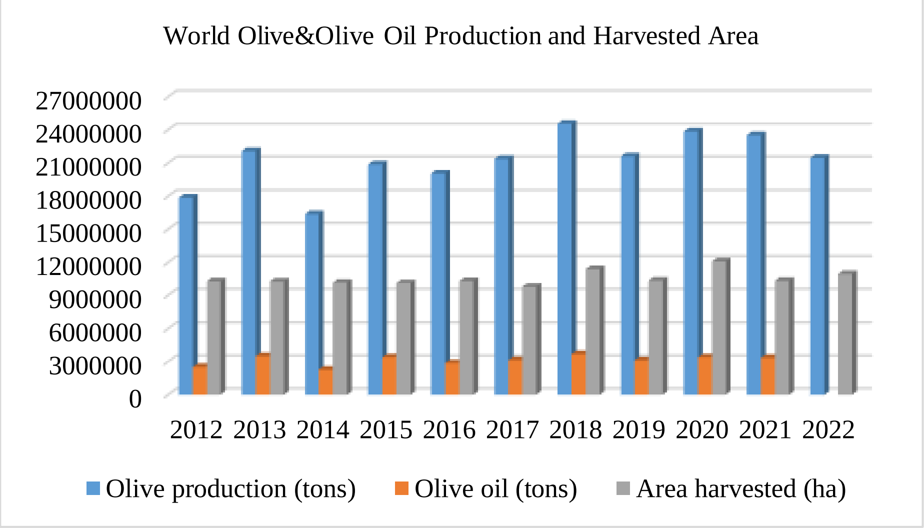
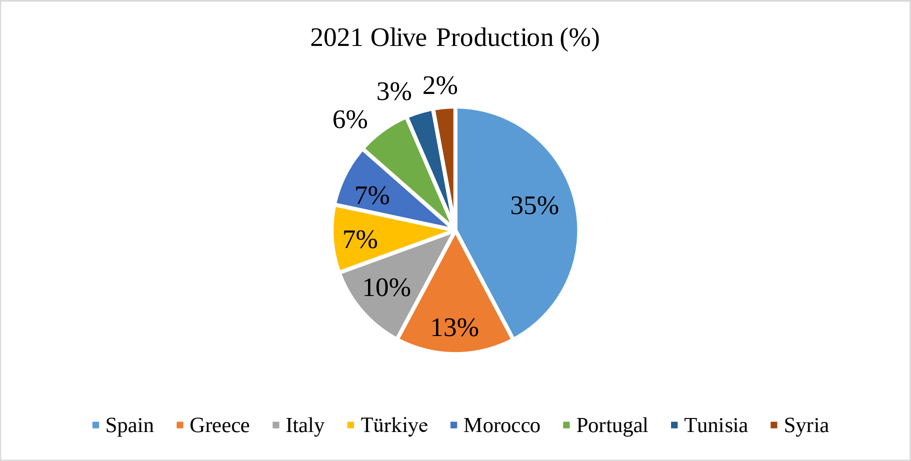


Figure 2. World Olive Hervested Area, Olive and Olive Oil Production between 2012 and 2022 (FAO, 2024)

Spain is the leading country in olive production, providing approximately 45% of the world's olive oil production. The share of Greece, Italy, Türkiye, Morocco, Portugal, Tunisia and Syria in production are 13%, 10%, 7%, 7%, 6%, 3%, and 2% respectively (Fig. 3).

It has been reported by the International Olive Council that olive oil consumption almost doubled from 1990/91 to 2020/21 (IOC, 2024). With the increasing demand for olive oil, olive has begun to be grown in different regions far from their countries of origin in recent years, especially in Japan, the USA, Australia, China, South America and South Africa (Rugini et al., 2011). In addition, the demand for "different and high-quality olive products" has increased considerably in recent years, especially in the national and international markets.



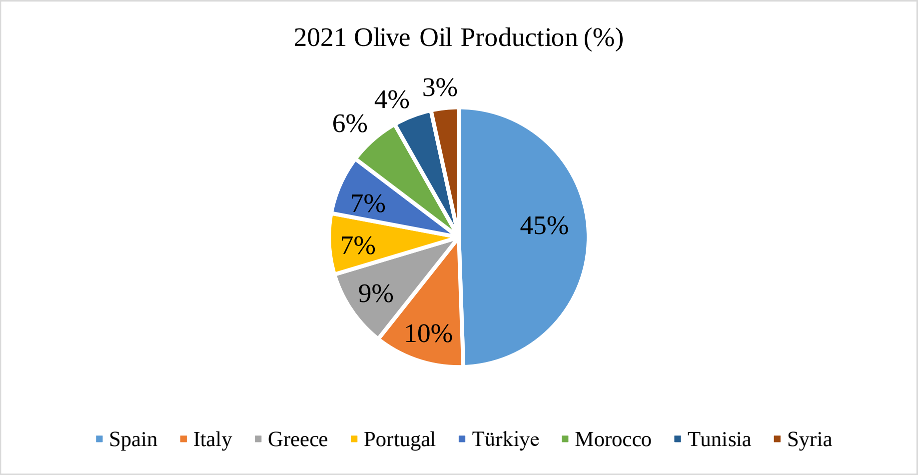


Figure 3. Percentages of Olive and Olive Oil Production by Countries in 2021 (FAO, 2024)

The US and the EU import almost 50% of the world's olive oil (34% and 15% respectively), while Brazil imports 9%, Japan and Canada 5%, China 4%, Australia and Russia 3%, and Mexico gets 2% (IOC, 2024).

4. TÜRKİYE OLİVE PRODUCTİON

Türkiye is a significant olive producing country. According to FAO 2022, Türkiyr ranks 4th in world olive production, 3rd in table olive production and 5th in olive oil production (FAO, 2024).

The Ministry of Agriculture has been providing significant support to the olive sector to increase olive production for the last 20 years. Thanks to these supports, the number of olive trees has increased considerably. While the number of trees was approximately 98 million in 2000, this number doubled to 195 million in 2022. Once all of these trees reach full productivity, it seems possible that the yield will increase over the years (Fig. 4).

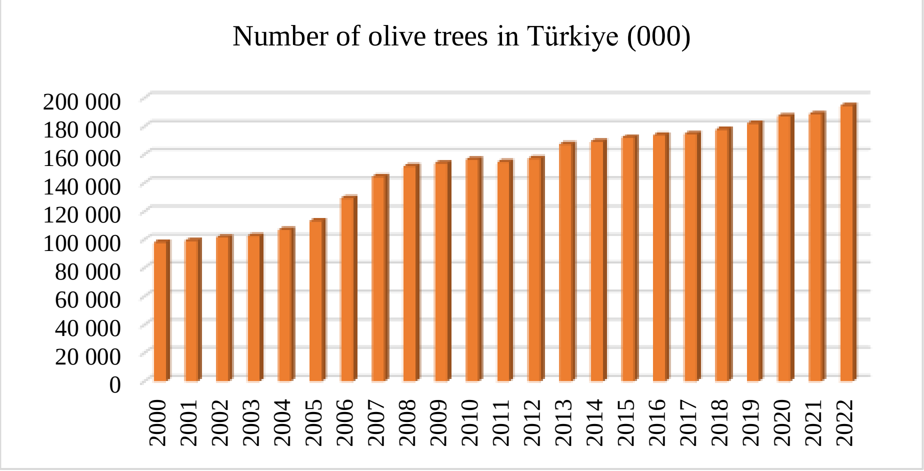


Figure 4. Number of Olive Trees in Türkiye (TUIK, 2023)

The olive growing area in Türkiye has reached 889,168 hectares and this area constitutes approximately 7% of the world's olive production areas (FAO, 2024). While our production amount was 1,738,000 tons in 2021, it increased by approximately 71% and reached 2,976 000 tons in 2022. These results reveal Türkiye's real potential in olive production in the full productive year. Türkiye's olive production in the last 23 years has averaged 1,853,000 tons. While the share of table olives in Türkiye's total production is 30%, 70% of the production is used for oil. (Fig. 5) (TUIK, 2023). Table olives and olive oil production is carried out in accordance with quality standards. And there are mandatory export standards for table olives and olive oil in Türkiye. In all facilities, work is carried out in accordance with the Turkish Standards Institute (TSE) standards, which are compatible with international standards (TMT, 2024).

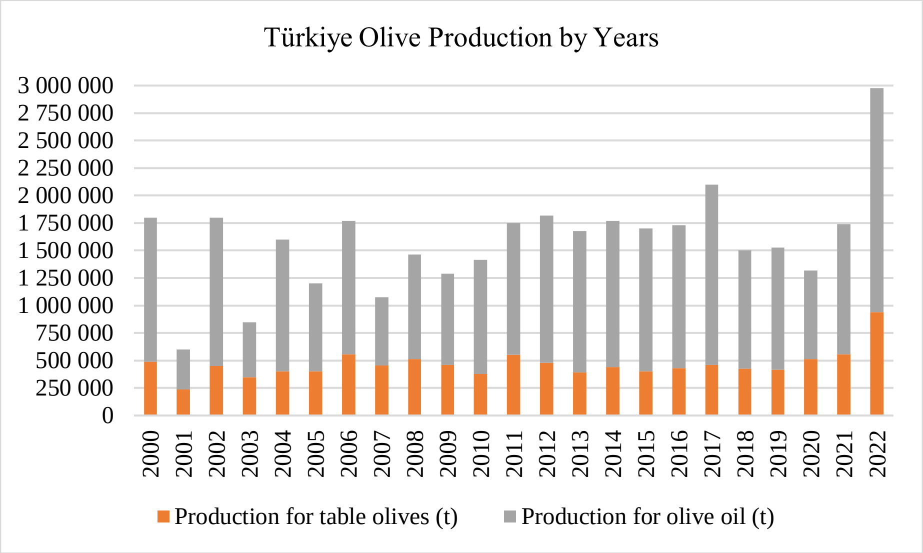


Figure 5. Olive Production in Türkiye (TUIK, 2023)

Olive oil consumption per inhabitant in the most important olive producing countries is shown in Fig. 6 (IOC, 2024). Although Türkiye is one of the main producing countries, consumption is at a very low level of 1.8 kg per person. Olive oil consumption is more common in coastal regions where olives are grown in Türkiye. In other regions, consumption culture is not fully formed. However, in recent years, awareness of the health benefits of olive oil, which is the most important element of the Mediterranean diet, has increased and olive oil consumption has begun to expand in other regions.

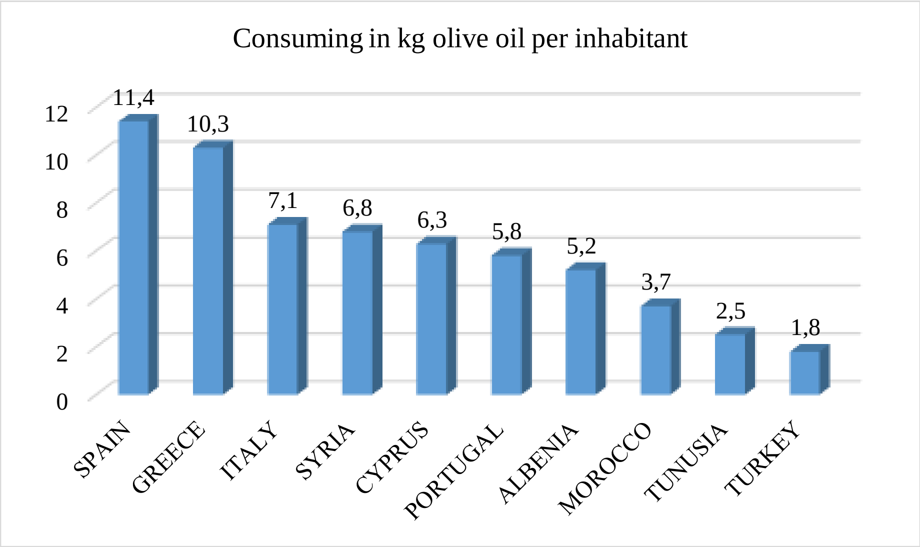


Figure 6. Kg olive oil consumption per inhabitant of the most important olive producing countries

5. SPREAD OF OLİVE CUTİVATİON IN TÜRKİYE

Türkiye is the 37th largest country in the world with a surface area of 780,043 km² (MSM, 2024). The country is surrounded by the Aegean Sea in the west, the Black Sea in the north and the Mediterranean Sea in the south. Additionally, the Marmara Sea is located in the northwest.

There are many macro-micro climate regions in Türkiye with very different characteristics such as climate, soil and topography. The climate of Türkiye has different temperature and precipitation regimes depending on the region (Tuğaç and Sefer, 2021). Due to these differences, Türkiye is among the origin regions of many species. Olive is one of the most important of these species. The crucial factor limiting olive cultivation is temperature. For this reason, olive cultivation is widespread, especially in coastal regions with a Mediterranean regime.

Figure 7. Monumental trees in Türkiye (original photos)

Aegean, Marmara, Mediterranean and Southeastern Anatolia Regions are the most important olive producing regions (Fig. 8) (TSMS, 2024). Especially the Aegean Region has approximately 54% of the total olive growing areas. More than half of the total olive production comes from this region. 23% of the olive production takes place in the Mediterranean region, 18% in the Marmara region, 6% in Southeastern Anatolia and 0.2% in the Black Sea Region. (TUIK, 2023).

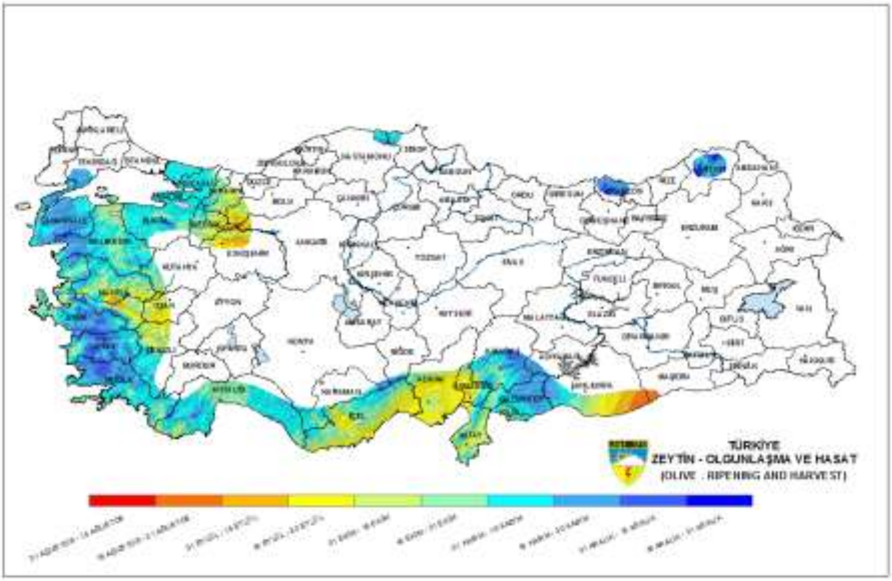


Figure 8. Olive plantation area with ripening and harvesting time.
(Source: Turkish State Meteorological Service).

The provinces with the most olive production in Türkiye are Aydın, İzmir, Muğla, Balıkesir, Bursa, Manisa, Çanakkale, Gaziantep and Mersin, respectively. The statistical data of these provinces between 2021 and 2023 are presented in Table 1 (TUIK, 2023). It has been shown that yield values per tree vary according to years in Table 1. After a year of great production, sometimes there may be a year with no or very low production. The tendency of cultivars to periodicity causes this fluctuation in yield. It can be seen mostly in traditional orchards. Because the periodicity tendency of cultivars can be reduced by practices such as fertilization, harvesting, pruning, irrigation and protection from pathogens applied in olive orchards with modern cultivation systems.

Table 1. Number of Trees, Production (t), Yield per Tree (kg) and Production Area (da) in the most important provinces of Türkiye, 2021-2023 (TUIK, 2023)

Province	Year	Total number of the olive trees	Total production area (da)	Production (t)	Average of the yield per tree (kg)
	2021	24,750,197	1,543,496	287,707	13
	2022	25,150,416	1,551,711	332,164	15,5
Aydın	2023	25,220,081	1,549,756	173,037	9
	2021	20,060,271	936,770	168,914	10
	2022	23,024,751	979,988	461,813	26,5
İzmir	2023	22,692,912	949,417	219,622	14,5
	2021	16,833,148	1,000,010	182,528	14,5
	2022	15,974,096	995,441	245,119	23
Muğla	2023	19,689,488	995,531	174,398	12,5
	2021	11,973,682	836,126	177,712	15,5
	2022	12,333,516	849,191	261,859	21
Balıkesir	2023	12,382,022	850,317	98,198	10,5
	2021	11,973,315	440,419	146,477	12
	2022	11,976,570	440,110	187,692	16
Bursa	2023	11,984,214	440,215	96,498	8
	2021	26,453,505	11,27,520	269,160	12
	2022	27,962,336	1,152,976	418,155	18,5
Manisa	2023	30,588,078	1,184,833	199,387	8
	2021	5,556,958	326,450	34,618	11
	2022	5,570,082	327,105	125,852	24,5
Çanakkale	2023	5,582,658	327,989	62,391	12
	2021	10,376,166	459,842	33,001	3,5
	2022	10,399,720	460,842	60,179	6,5
Gaziantep	2023	10,415,910	461,442	73,951	8
	2021	13,953,769	425,734	104,952	9,5
	2022	13,398,285	397,187	356,279	32,5
Mersin	2023	13,674,612	398,069	95,451	9

The share of olive growing areas in Türkiye's total agricultural area is approximately 3%. Olive cultivation in Türkiye has a traditional structure. Production areas are generally small-scale areas such as 5-10 decares. The average yield per tree is 12-13 kg. In traditional cultivation, there are approximately 10-20 trees per decare (Ozaltas et al., 2016). Additionally, traditional olive orchards take place in Türkiye are located especially in marginal regions, and these areas constitute a large portion of the total olive orchards. For example, approximately 30% of the olive orchard located near

Aydın, Manisa and İzmir have extremely sloped areas (26% and above), while 20% are located in moderately sloped areas (16-25%) and 22% in areas with low slope. The proportion of areas where the slope is flat or nearly flat (0-5%) is approximately 27% (Oztürk et al., 2021). Olive cultivation in Türkiye is usually conducted in these three provinces (Table 1).

On the other hand, in order to increase olive cultivation in Türkiyr, significant agricultural supports have been provided by the Ministry of Agriculture, especially since 2005. With these supports and the opening of non-agricultural areas to olive cultivation, there has been a significant increase in the number of olives. olive trees (Ozaltaş et al., 2016).

6. THE MOST COMMON OLIVE CULTİVARS IN THE OLIVE GROWING COUNTRIES

Genetic diversity in olives is quite high (Fanelli et al., 2022), due to reasons such as beginning of olive cultivation in the early period, the use of vegetative propagation methods, and the continuation of mutations for centuries (Bellini et al., 2008). As a result, the number of cultivars of olives is very high all over the world.

It has been reported by many researchers that the number of olive cultivars, including homonyms and synonyms, is over 2000 (Galatali et al., 2021; Fanelli et al., 2022; Acar, 2024). Olive cultivars are protected in 54 countries and more than 100 collections and are included in the FAO olive genebank database (Bartolini 2008; Bracci et al., 2011). Most of these cultivars are located in Southern European countries. The number of cultivars is reported to be over 600 in Italy (Sion et al., 2019), 183 in Spain, 88 in France, and 52 in Greece (Baldoni & Belaj, 2009; Bracci et al., 2011). The number of national cultivar in Türkiye has reached 102 with the latest registration studies (Acar, 2024; TTSM, 2024).

SPAIN:

Spain is the largest olive producer country in the World. Olive cultivation is distributed in 35 of Spain's 50 provinces and mostly located in the south, southeast and east of the peninsula. Among the largest olive regions in Spain is Andalusia, which accounts for 59.98%; This is followed by Extremadura (10.51%), Catalonia (4.46%), Valencia (3.66%), Aragon (2.28%) and Castille la Mancha (1.84%) (Millán-Vazquez de la Torre et al., 2017). As a result of the survey conducted in Spain, 262 different cultivars were identified and 24 of them were considered as major cultivars that are more common in olive growing regions. ‘Picual’ is the main cultivar and has been particularly preferred due to its many positive characteristics such as early bearing, high yield and low fruit removal force that facilitates mechanical harvesting. 'Arbequina' is also preferred for intensive planting areas due to its low vigor, early bearing and high productivity and high olive content. Other outstanding major cultivars are ‘Cornicabra’, ‘Hojiblanca’, ‘Lechín de Sevilla’ and ‘Manzanilla de Sevilla'. ‘Manzanilla' is a world famous cultivar used as table olives (Barranco et al., 2000).

ITALY:

One of the other important olive producing countries is Italy. Different olive cultivars grow depending on the region in Italy. ‘Carolea’ and ‘Nocellara’ cultivars grow in Calabria; ‘Frantoio’, ‘Carolea’, ‘Coratina’, ‘Leccino’ and ‘Ogliarota’ in Campagna; ‘Frantoio’ in Latium, ‘Castelvetrano’ in Sicily, ‘Opalino’ and ‘Taggiasca’ in Liguria and ‘Cerignolas’ in different regions (Efe et al., 2013; Ozturk et al., 2021).

GREECE:

In Greece, which ranks third in olive production after Spain and Italy, 20 of the 40 existing cultivars (excluding clonal selections) cover 90% of the growing area (Hagidimitriou et al., 2005). These are: ‘Agouromanakolia’, ‘Adramitini’, ‘Amigdalolia’, ‘Asprolia’, ‘Valanolia’, ‘Vasilikada’, ‘Gaidurelia’, ‘Dafnelia’, ‘Thiaki’, ‘Kalamon’, ‘Kalokerida’, ‘Karolia’, ‘Karidolia’, ‘Kothreiki’, ‘Kolimpada’, ‘Konservolia’, ‘Koroneiki’, ‘Koutsourelia’, ‘Lianolia Kerkiras’, ‘Mastoeidis’ (referred also as ‘Athinolia’ or ‘Tsounati’), ‘Mavrelia’, ‘Megaritiki’, ‘Mittolia, ‘Strogilolia’, ‘Throumbolia’, and ‘Tragolia’ (Kalogeropoulos and Tsimidou, 2014). Oil production in Greece relies heavily on the 'Koroneiki' cultivar.

MOROCCO:

In Morocco, a single cultivar, named Picholine marocaine, is grown intensively in olive orchards. Only a few other cultivars (Bouchouk, Bouchouika, Fakhfoukha, Hamrani and Meslala) are known, and they are grown in limited areas (Khadari et al., 2008).

PORTUGAL:

In Portugal, 'Galega vulgar' cultivar is the main national cultivar, widespread throughout the country. It is mostly used for olive oil production. It is characterized by high vigour, low-weight fruits (< 2 g), resistance to drought, and excellent quality olive oil (Sales et al., 2024).

7. THE MOST COMMON OLIVE CULTİVARS IN THE TURKİYE

Until recently, different olive cultivars spread to different regions in Türkiye. It is possible to call these regions olive cultivar regions. Most of the existing trees consisted of one or several cultivars in different region. For example, ‘Gemlik’ is the most common cultivar in the Southern Marmara, ‘Ayvalık’ cultivar in the Northern Aegean, and ‘Memecik’ cultivar in the Southern Aegean. However, in the last 15-20 years, olive seedling production with cutting has become widespread. Cultivars that can be easily propagated by cuttings, such as ‘Gemlik’ and ‘Ayvalık’, have started to be planted in all olive regions. In 1991, the ratio of cultivars grown in Türkiye to the total number of trees was reported as 45.5% in ‘Memecik’cultivar, 19% in ‘Ayvalık’, 11% in ‘Gemlik’, 3.5% in ‘Erkence’, 2.8% in ‘Kilis Yağlık’, 2.2% in ‘Halhalı’, 2% in ‘Nizip Yağlık’, 1.55% in ‘Çekişte’. 1.4% in the ‘Domat’ cultivar, 1% in ‘Uslu’ cultivar, 0.7% in the ‘Sarı Haşebi’ cultivar, 0.6% in the ‘Sarı Ulak’ cultivar, 0.6% in the ‘Saurani’ cultivar, and 8.2% in other cultivars (Canözer, 1991). However, in a study conducted 15 years later, the percentage distribution of cultivars in olive regions was determined and it was reported that 48.71% of the total olive tree consists of ‘Gemlik’ cultivar, 20.66% ‘Ayvalık’, 19.11% ‘Memecik’, 7.56% ‘Domat’ and 3.73% other olive cultivars (Ozaltaş et al., 2016).

The fact that almost half of our production consists of a single cultivar (Gemlik c.v.) means that existing genetic resources are not fully utilized. This situation may cause negative effects in the long term. Because when we observe the effects of possible climate change, the importance of resistant cultivars will become more important. It is necessary to introduce cultivars that can easily adapt to extreme environmental conditions such as hot and cold, resistant to some diseases and pests, as well as cultivars that stand out in terms of quality and yield, and to increase the number of cultivars grown.

In fact, this situation is similar in the world. Although there are around 2000 different cultivars in the world, it has been reported by the International Olive Council (IOC) that only 139 of these cultivars constitute approximately 85% of the world olive production (IOC, 2024).

There are 102 national cultivar registered in Türkiye so far. Some of these cultivars grown in the Marmara, Aegean, Mediterranean, Southeastern Anatolia and Black Sea regions are presented in Table 2.

Table 2. Some common cultivars by region in Türkiye

Region	Cultivar name
Marmara Region	Gemlik
	Edincik Su
	Karamürsel Su
	Çelebi
	Samanlı
Aegean Region	Ayvalık
	Memecik
	Domat
	Erkence
	Çekişte
	Uslu
	Çilli
Mediterranean Region	Saurani
	Sarı ulak
	Halhalı (Hatay)
	Srı Ulak
	Büyük Topak Ulak
Southeastern Anatolia Region	Nizip Yağlık
	Kilis Yağlık
	Halhalı (Derik)
	Eğri burun
Black Sea Region	Butko
	Patos
	Otur
	Görvele

<u>Gemlik:</u>

Origin: Gemlik/Bursa

Synonym: Trilye, Kaplık, Kıvırcık, Kara

‘Gemlik’ cultivar constitutes approximately half of Türkiye's olive trees. Its origin is the Marmara Region. Most of the black table olives are produced in the Marmara Region. Approximately 80% of the table production consists of the Gemlik cultivar.

'Gemlik' has a medium vigor tree structure. It has started to be grown in all olive regions of Turkey due to its high oil content (29%), easy rooting from cuttings, low periodicity tendency and its suitability especially for black table production (Kaya et al., 2015).

‘Gemlik’ cultivar is promoted as the best Turkish cultivar due to its relatively high oil yield, early ripening, unique organoleptic and sensory properties, and suitability for processing natural black olives in brine (Keceli et al., 2024).

<u>Ayvalık:</u>

Origin: Edremit/Balıkesir

Synonym: Edremit yağlık, Şakran, Midilli, Ada Zeytini

‘Ayvalık’ is the second most common cultivar in Türkiye. Its origin is the northern Aegean region. It constitutes a quarter of the tree population in the Aegean Region. It is also grown in other regions such as Antalya, Mersin, and Adana (Ucuncuoglu and Ozay, 2020).

It has a strong and upright tree structure. It can be easily propagated by cutting. Although it is generally used as oil (>22%), it is also a preferred cultivar used for green, pink and black table purposes (Kaya et al., 2015).

<u>Memecik:</u>

Origin: Mugla

Synonym: Ası yeli, Gülümbe, Sehir, Tekir, Tas arası, Yaglık

'Memecik' cultivar constitutes the majority of the trees in the southern Aegean region. It is the most common variety, especially in the most important olive regions such as Aydın and Muğla.

It has a strong and spreading tree structure and drought resistant. But it shows high periodicity. It is an important olive vcultivar in terms of its high oil content (>22%) and the chemical and sensory properties of its oil. Therefore, it is generally used for oil purposes. And it can also be used for the production of green and black table olives (Kaya et al., 2015).

<u>Domat:</u>

Origin: Akhisar/Manisa

'Domat' cultivar is grown in the Aegean region and orginates from Akhisar district. It is one of the most common green table cultivar in Türkiye. It is suitable for different table processing techniques such as filling or grilling.

‘Domat’ is cultivated in Manisa, Akhisar, Turgutlu, Saruhanlı, İzmir, Kemalpaşa, Selçuk, Söke, Karacasu and Kuyucak (Efe et al., 2013).

Tree forms of the Domat cultivar are quite large, strong and spreading. Its juvenile period is short and its productivity is high. The oil content is low and the fruits are quite large (Kaya et al., 2015).

<u>Erkence:</u>

Origin: İzmir

Synonym: İzmir yaglık, Yerli yaglık, Hurma zeytin

'Erkence' cultivar orginates from İzmir. It is grown in the Aegean region and very old trees in Izmir are generally of this cultivar.

'Erkence' cultivar is one of the earliest ripening cultivar from Türkiye. It has strong and spreading tree habit. The oil content of Erkence’ is high (>22%). It is generally used for olive oil purposes. However, due to the debittering properties of its fruits, it is also consumed as 'Hurma olive' (Kaya et al., 2015).

Hurma olive is defined as an olive product that removes the bitter taste of the olive while it is still on the tree as a result of debittering. For this reason, this fruits can edible. “Debittering” is the term expressed as the maturation period occurring in the olive fruit while it is still on the tree (Susamci et al., 2016).

<u>Kilis Yağlık:</u>

Origin: Kilis

'Kilis Yağlık' is the most common cultivar grown in the Southeastern region of Türkiye, representing approximately 52% of the growing area (Arslan and Ozcan, 2014). It is cultivated especially in the Kilis Gaziantep, Şanlıurfa, Kahramanmaraş ve Mardin.

The growth habit of the trees is medium and spreading. It has a very high oil content (30%). It produces fruits of all sizes on the same tree (Kaya et al., 2015). This is not a desired feature.

Nizip Yağlık:

Origin: Nizip

'Nizip Yağlık' is the second important cultivar of the Southeastern Anatolia region. It is cultivated in Kahramanmaraş, Mardin and Cizre.

Tree forms of this cultivar are medium and spreading. Oil content is high (>22%). Although it is generally used for oil purposes, it is also consumed as black table olive

Halhalı (Derik):

Origin: Derik

It is cultivated in the Southeastern Anatolia region, especially in Hatay, Gaziantep, Mardin and Kahramanmaraş provinces.

The growth habits of the trees are medium and spreading.

It has a high oil content. It shows periodicity. The fruits are very small. But it is also consumed as green crushed olives (Kaya et al., 2015).

Butko:

Origin: Artvin

It iscultivated in the Çoruh Valley in the Black Sea region. It is used for both purposes as oil and table olives. The tree habit is weak and spreading. The oil content is moderate (18-22%) (Kaya et al., 2015).

Sarı Ulak:

Origin: Tarsus-Mersin

'Sarı Ulak' is an important cultivar of the Mediterranean region. It is cultivated in the Erdemli, Gülnar, Tarsus, Seyhan, Kozan and Yumurtalık. It constitutes 6% of the olive trees in the Mediterranean region (Efe et. al., 2013).

The tree habit is strong and spreading. The oil content is moderate (18-22%). It is generally produced as a green and black table olives. (Kaya et al., 2015).

Morover, many exploting survey studies have been conducted in different regions within the scope of collecting and protecting genetic resources in Türkiye. As a matter of fact, the "Hanım Parmagı" cutlivar discovered through these survey studies belongs to the Çanakkale region and was registered in 2017; 'Beylik' cultivar from Antalya region registered in 2020; ‘Ada Yerlisi' cultivar was registered from Cyprus in 2024 (TTSM, 2024).

In addition, crossbreeding programs with different varieties for different purposes are ongoing from Izmir Olive Reseacrh Institute. As a result of these studies, the 'Hayat' cultivar in 2015 and Arsel' cultivar in 2019 were registered as Turkiye's first hybrid varieties in terms of table olive characteristics (TTSM, 2024).

8. CONCLUSION

Olive is an important agricultural product that has been grown in a wide geography for centuries due to its adaptability and resistance to extreme conditions, and its importance and interest level has increased over time.

Since olive also affects many sectors it is a product of socioeconomic importance. It affects many segments of the population dealing with national and international trade, from farmers to workers in rural areas, from olive producers to the olive oil and table olive industry.

Olive is also one of the priority and important study subjects of national and international scientists, which includes many issues such as ensuring sustainability, developing new cultivars resistant to possible climate changes, protecting existing genetic resources, and fully identifying and effectively using these rich genetic resources. In this context, many national and international projects are carried out in Türkiye. Existing genetic resources are protected and new cultivars are added to these resources.

REFERENCES

Acar, S. (2024). General purpose and selection criteria of olive breeding programs. Ahi Evran International Congress on Scientific Research-IV, April 26-28, 2024 / Full Texts Book, Volume 1, 493-503. ISBN - 978-625-367-709-1. https://www.ahievranconference.org/books

Arslan D, Özcan M. (2014). Changes in Chemical Composition and Olive Oil Quality of Turkish Variety ’Kilis yağlık’ with Regard to Origin of Plantation. Glob. J. Agric. Innov. Res. Dev., 1(2):51-6. https://doi.org/10.15377/2409-9813.2014.01.02.3

Baldoni, L., Belaj, A. (2009). Olive. In: Vollmann, J., Rajcan, I. (eds) Oil Crops. Handbook of Plant Breeding, vol 4. Springer, New York, NY. https://doi.org/10.1007/978-0-387-77594-4_13

Baranco, D., Rallo, L. (2000). Olive Cultivars in Spain. HortTechnology, 10, 107–110. http://dx.doi.org/10.21273/HORTTECH.10.1.107

Bartolini, G. (2008). Olea databases. Accessed: 20.04.2024. http://www.oleadb.it

Bellini, E., Giordani, E., & Rosati, A. (2008). Genetic improvement of olive from clonal selection to cross-breeding programs. Advances in Horticultural Science, 22(2), 73–86. http://www.jstor.org/stable/42882621

Besnard, G., Rubio de Casas, R. (2015). Single vs multiple independent olive domestications: the jury is (still) out. New Phytol. 2016 Jan;209(2):466-70. doi: 10.1111/nph.13518. Epub 2015 Nov 11. PMID: 26555218. https://doi.org/10.1111/nph.13518

Besnard, G., Khadari, B., Navascue´s, M., Ferna´ndez-Mazuecos, M., El Bakkali, A., Arrigo, N., Baali-Cherif, D., Brunini-Bronzini de Caraffa, V., Santoni, S., Vargas, P., Savolainen, V. (2013). The complex history of the olive tree: from Late Quaternary diversification of Mediterranean lineages to primary domestication in the northern Levant. Proc R Soc B 280: 20122833. http://dx.doi.org/10.1098/rspb.2012.2833

Bracci, T., Busconi, M., Fogher, C., Sebastiani, L. (2011). Molecular studies in olive (Olea europaea L.): overview on DNA markers applications and recent advances in genome analysis. Plant Cell Report 304494620721-7714. https://doi.org/10.1007/s00299-010-0991-9

Canözer, O., 1991. Standart Zeytin Çeşitleri Kataloğu, Tarım ve Köyişleri Bakanlığı, Yayın Dairesi Başkanlığı, Mesleki Yayınlar, Seri:16, No:334, Ankara, 41-43s.

Carrión, Y., Ntinou, M., Badal, E. (2010). Olea europaea L. in the north Mediterranean Basin during the Pleniglacial and the early–middle Holocene. Quaternary Science Reviews 29: 952–968. https://doi.org/10.1016/j.quascirev.2009.12.015

Chiappetta, A., & Muzzalupo, I. (2012). Botanical Description. Olive Germplasm - The Olive Cultivation, Table Olive and Olive Oil Industry in Italy. InTech. doi: 10.5772/51836. http://doi.org/10.5772/3314

Cimato, A.; Attilio, C. (2011). Worldwide diffusion and relevance of olive culture. In Olive Diseases and Disorders; Schena, L., Agosteo, G.E.,Cacciola, S., Eds.; Transworld Research NetWork: Trivandrum, India, 2011; pp. 1–21. https://www.researchgate.net/publication/274553418

Cronquist, A., (1981). An Integrated System of Classification of Flowering PlantsColumbia University Press, New York, 0-23103-880-1.

Efe, R.; Soykan, A.; Cürebal, I.; Sönmez, S. (2013). Olive and Olive Oil; Edremit Municipality: Balıkesir, Turkey; ISBN 978-605-62253-3-8.

Erel, R., Yermiyahu, U., Van Opstal, J., Ben-Gal, A., Schwartz, A., Dag, A. (2013). The importance of olive (*Olea europaea* L.) tree nutritional status on its productivity. Scientia Horticulturae, 159, 8-18. https://doi.org/10.1016/j.scienta.2013.04.036

Falek, W., Mascio, I., Gadaleta, S., Fanelli, V., Bechkri, S., Khelifi, D., Miazzi, M.M., Montemurro, C. (2022).Morphological and Eco-Geographic Variation in Algerian Wild Olives. Plants, 11, 1803. https://doi.org/https://doi.org/10.3390/plants11141803

Fanelli, V.; Mascio, I.; Falek, W.; Miazzi, M.M.; Montemurro, C. (2022). Current Status of Biodiversity Assessment and Conservation of Wild Olive (*Olea europaea* L. subsp. *europaea* var. *sylvestris*). Plants, 11(4), 480. https://doi.org/10.3390/plants11040480

FAO (2024). FAOSTAT Statistics. http://www.fao.org/faostat/en/#data/QC. Accessed: 15.05.2024

Galatali, S., Ghafoor, N.A., Kaya, E. (2021). Characterization of Olive (*Olea Europaea* L.) Genetic Resources via PCR-Based Molecular Marker

Systems. European Journal of Biology and Biotechnology, 2(1), 26-33. https://doi.org/10.24018/EJBIO.2021.2.1.146

Green, P. S. (2002). A Revision of Olea L. (Oleaceae). Kew Bulletin, 57(1), 91–140. https://doi.org/10.2307/4110824

Hagidimitriou, M., Katsiotis, A., Menexes, G., Pontikis, C., Loukas, M. (2005). Genetic diversity of major Greek olive cultivars using molecular (AFLPs and RAPDs) markers and morphological traits. J. Am. Soc. Hortic. Sci., 130, 211–217. https://doi.org/10.21273/JASHS.130.2.211

IOC (2024). International Olive Oil Council. https://www.internationaloliveoil.org . Accessed: 15.05.2024

Kalogeropoulos, N., Tsimidou, M.Z. (2014). Antioxidants in Greek Virgin Olive Oils. Antioxidants, 3, 387-413. https://doi.org/10.3390/antiox3020387

Kaya, H., Sefer, F., Mete, N., Çetin, Ö., Hakan, M., Şahin, M., Güroğlu, U., Uluçay, N., Veral, M.G. (2015) Türkiye Zeytin Çeşit Kataloğu. Zeytin Araştırma Enstitüsü Müdürlüğü, ISBN: 978-605-9175-04-3. Bornova/İzmir. 197s

Keceli, T.M., Celik, F.H., Koseoglu, O. (2024). Effect of Growing Regions on Discrimination of Turkish-Style Black Table Olives from Gemlik Cultivar. Japan Science and Technology Agency, 73(3), 321-331. https://doi.org/10.5650/jos.ess23057

Khadari, B., Charafi, J., Moukhli, A., Ater, M. (2008). Substantial genetic diversity in cultivated Moroccan olive despite a single major cultivar: a paradoxical situation evidenced by the use of SSR loci. Tree Genetics & Genomes 4, 213–221. https://doi.org/10.1007/s11295-007-0102-4

Langgut, D., Cheddadi, R., Sebastian Carrion, J., Cavanagh, M., Colombaroli, D., Eastwood, W. J., Greenberg, R., Litt, T., Mercuri, A. M., Miebach, A., Roberts, C. N., Woldring, H., & Woodbridgel, J. (2019). The origin and spread of olive cultivation in the Mediterranean Basin: The fossil pollen evidence. Holocene, 29(5), 902-922. https://doi.org/10.1177/0959683619826654

Langgut, D., Garfinkel, Y. (2022). 7000-year-old evidence of fruit tree cultivation in the Jordan Valley, Israel. Sci Rep 12, 7463. https://doi.org/10.1038/s41598-022-10743-6

Lanza, F. (2012). Olive: A Global History. Reaktion Books, ISBN: 9781861899729. https://books.google.com.tr/books?id=YiGZtRj95-kC

Marie, C., Warnock, P., Jean, A. (2012). Origin and History of the Olive. http://doi.org/10.5772/51933

Millán-Vazquez de la Torre, M.G., Arjona-Fuentes, J.M., Amador-Hidalgo, L. (2017). Olive oil tourism: Promoting rural development in Andalusia (Spain), Tourism Management Perspectives, Tourism Management Perspectives, 21, 100-108. https://doi.org/10.1016/j.tmp.2016.12.003

MSM (2024). Ministry of National Defense, General Directorate of Mapping. https://www.harita.gov.tr/il-ve-ilce-yuzolcumleri Accessed: 10.06.2024

Nteve, G.-M., Kostas, S., Polidoros, A.N., Madesis, P., Nianiou-Obeidat, I. (2024). Adaptation Mechanisms of Olive Tree under Drought Stress: The Potential of Modern Omics Approaches. Agriculture, 14, 579. https://doi.org/10.3390/agriculture14040579

Ozaltaş, M., Savran, M.K., Ulaş, M., Kaptan, S., Köktürk, H., Kalanlar, Ş., Konsoloğlu, B., Tibet, Ü., Yağcıoğlu, M., Aksarı, E., Tatlı, A., Soyuer, H., Pekcan, T., Dursun, Ö., Karadeniz, C., Kilci, M., Balkan, A., Sevilmiş, G. (2016). Türkiye Zeytincilik Sektör Raporu, Zeytincilik Araştırma Enstitüsü Müdürlüğü, Bornova, İzmir. https://www.researchgate.net/publication/311421869_Turkiye_Zeytincilik_Sektor_Raporu_Turkish_Olive_Sector_Report#fullTextFileContent

Ozturk, M., Altay, V., Gönenç, T.M., Unal, B.T., Efe, R., Akçiçek, E., Bukhari, A. (2021). An Overview of Olive Cultivation in Turkey: Botanical Features, Eco-Physiology and Phytochemical Aspects. Agronomy, 11, 295. https://doi.org/10.3390/agronomy11020295

Rugini, E., De Pace, C., Gutiérrez-Pesce, P., Muleo, R. (2011). Olea. In: Kole, C. (eds) Wild Crop Relatives: Genomic and Breeding Resources. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-16057-8_5

Sales, H., Alves, M.A., Sampaio, A.M., Nunes, J., Patto, M.C.V. (2024). Understanding the phenotypic variability of the olive 'Galega vulgar' fruits and oil-related traits across environments. Scientia Horticulturae, 326, 112738. https://doi.org/10.1016/j.scienta.2023.112738

Sion, S., Taranto, F., Montemurro, C., Mangini, G., Camposeo, S., Falco, V., Gallo, A., Mita, G.,Saddoud Debbabi, O., Ben Amar, F., Pavan, S., Roseti, V., Miazzi, M.M. (2019). Genetic Characterization of Apulian Olive Germplasm as Potential Source in New Breeding Programs. Plants, 8(8), 268. http://dx.doi.org/10.3390/plants8080268

Susamci, E., Ozturk Gungor, F., Irmak, S., Ataol Olmez, H., & Tusu, G. (2016). A study on the nutritional value of Hurma olives (Erkence cv.) that lose the bitterness on the tree. Journal of Agricultural Sciences, 22(4). http://dx.doi.org/10.24323/akademik-gida.474933

TMT, (2024). Olive oil and table olives. Republic of Türkiye – Ministry of Trade. Accessed: 10.06.2024. https://www.trade.gov.tr/data/5b8fd55613b8761f041fee87/Olive%20Oil%20and%20Table%20Olives.pdf

TSMS, (2024). Turkish State Meteorological Service. Olive plantation area with ripening and harvesting time. Accessed: 25.06.2024 https://www.mgm.gov.tr/veridegerlendirme/fenolojik-normal-haritalari.aspx?b=zeytin

TTSM (2024). Republıc of Türkiye Ministry of Agriculture and Forestry Cultivar Registratıon and Seed Certification Center. Accessed: 25.06.2024.https://www.tarimorman.gov.tr/BUGEM/TTSM/Sayfalar/Detay.aspx?SayfaId=87

Tuğaç, M. G., Sefer, F. (2021). GIS-based multi-criteria land suitability analysis for determining olive (*Olea Europaea* L.) cultivation areas in Turkey. Journal of Agriculture Faculty of Ege University, 58(1), 97-113. https://doi.org/10.20289/zfdergi.678474

TUİK (2023). Turkish Statistical Institute Data. https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2023-49535 .

Ucuncuoglu, D. and Ozay, D.S. (2020). Geographical origin impact on volatile composition and some quality parameters of virgin olive oils extracted from the “Ayvalık” variety. Heliyon, 6(9), E04919. https://doi.org/10.1016/j.heliyon.2020.e04919

Zeder M.A., Emswhiller E., Smith B.D., Bradley D.G. (2006). Documenting Domestication, the intersection of genetics and archaeology trends in genetics, 22: 139155. https://doi.org/10.1016/j.tig.2006.01.007

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