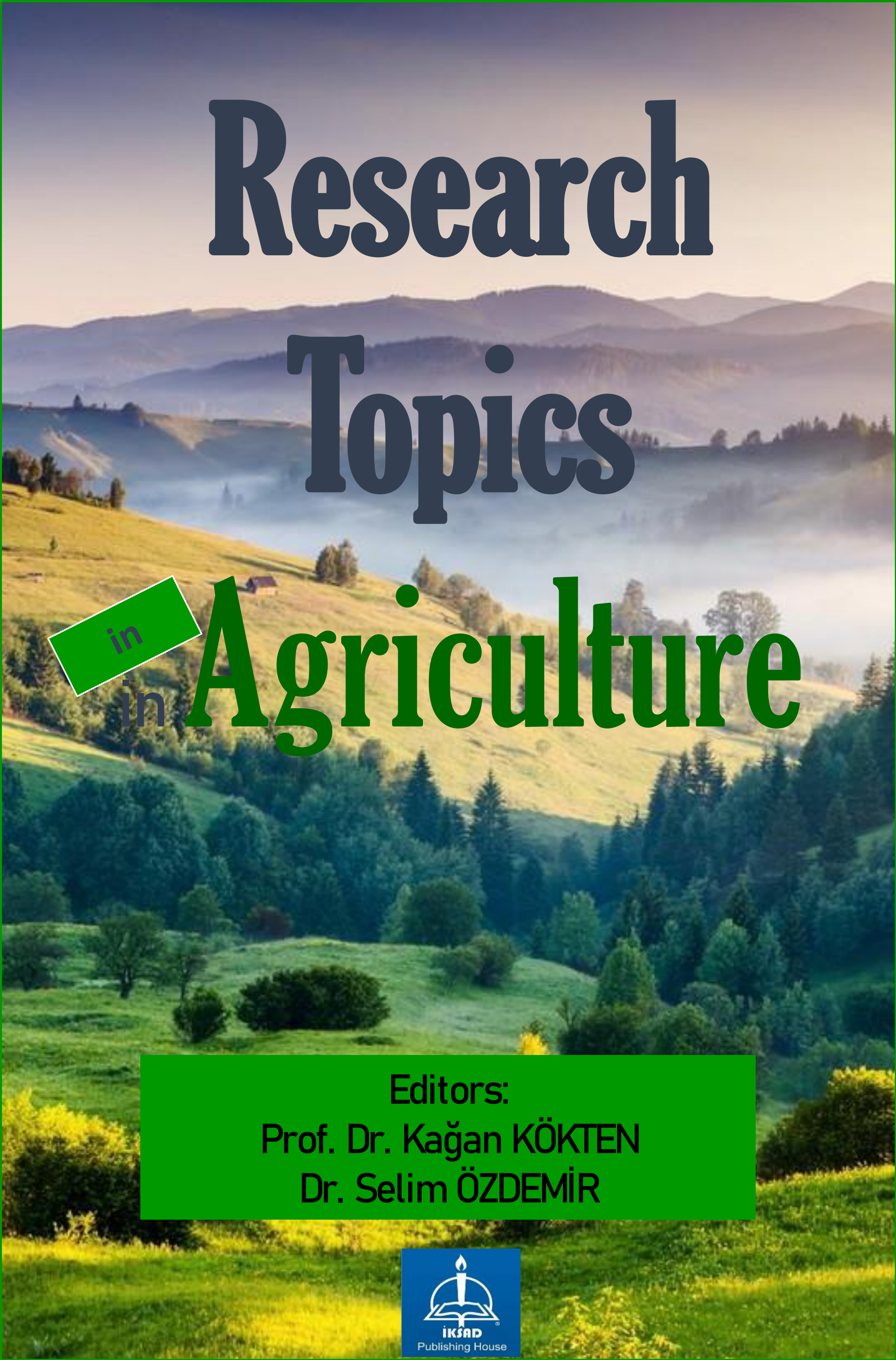

Research Topics in Agriculture

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Prof. Dr. Kağan KÖKTEN
Dr. Selim ÖZDEMİR

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RESEARCH TOPICS IN AGRICULTURE

EDITORS
Prof. Dr. Kağan KÖKTEN
Dr. Selim ÖZDEMİR

AUTHORS
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Prof. Dr. Füsun TEMAMOĞULLARI
Prof. Dr. İbrahim ATIŞ
Prof. Dr. Kağan KÖKTEN
Assoc. Prof. Dr. Hülya TORUN
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PhD Student Vesile YALCIN

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Researches Publications®
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TURKEY TR: +90 342 606 06 75
USA: +1 631 685 0 853
E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

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PREFACE

The majority of the world's food supply is provided by agriculture, which also maintains a vital number of ecosystem services. Agriculture is extremely essential for ensuring food security and advancing the Sustainable Development Goals. In order to attain the goal, we must address new areas for study agriculture related to climate change adaptation. In various parts of the world, studies have been published with various study focuses on raising food and nutritional security from an agricultural system perspective. The diversity of agricultural research studies necessitates an interdisciplinary and thorough systematisation of the various research objectives and the wide range of methodologies, analytical scales, and reference data employed. These include the top-level planning of priority development of agriculture and rural areas, linking mechanisms of poverty alleviation and rural revitalization, enhancing the quality and sustainability of agricultural products, realising the unity of food security and modern efficient agriculture, choosing the best rural reform, developing deep processing of county agricultural products, response to global hunger, genetically modified crops, contribution of technological progress to agriculture, organic farming & food, sustainable agricultural practices, pesticides & food quality, best practices in pest management, plant breeding, proper use of natural resources, innovative data-driven farming, vertical farms, biodiversity, food cultures, insects farming, agricultural supply chains & local food production.

Prof. Dr. Kağan KÖKTEN
Dr. Selim ÖZDEMİR

CHAPTER 1

ADVANTAGES OF INTERCROPPING SYSTEMS IN SUSTAINABLE AGRICULTURE

Prof. Dr. Kağan KÖKTEN[1]
Dr. Selim ÖZDEMİR[2*]

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[1] Sivas University of Science and Technology Faculty of Agricultural Sciences and Technology Department of Plant Production and Technology, Sivas, kkokten@sivas.edu.tr, Orcid ID: https://orcid.org/0000-0001-5403-5629

[2] Bingöl University, Vocational School of Food, Agriculture and Livestock, Bingöl, Corresponding author sozdemir@bingol.edu.tr, Orcid ID: https://orcid.org/0000-0003-1840-9907

Introduction

Throughout history, agriculture has been the foundation of economic, social, political, and cultural development, as it serves the purpose of meeting human needs for food and clothing by utilizing water resources, land, and energy. The agricultural sector, which is one of the most significant economic activities in various communities, requires consistent planning to promote development and cope with crises. Sustainable agriculture is an agricultural approach based on both environmental and social sustainability principles. This approach aims to carry out food production and agricultural practices in order to conserve natural resources, sustain ecosystems, support biological diversity, preserve soil health, combat climate change, and promote human health. In other words, sustainable agriculture should be ecologically sound, economically viable, and socially desirable. The goals of sustainable agriculture are closely related to their definitions. The objectives of a successful sustainable agriculture program are as follows: ensuring food security by increasing both quality and quantity while considering the needs of future generations; conserving water, soil, and natural resources; preserving energy resources within and outside farm activities; maintaining and improving farmers' profitability; sustaining the vitality of rural communities; and preserving biological diversity (Eskandari, 2012; Earles, 2005; Gruhn et al., 2000).

In traditional farming and monocropping systems, although the high yield obtained per unit area can meet the nutritional needs of the growing population in some regions, these systems require direct and indirect energy produced from costly fossil resources. Ecologically and environmentally, monocropping has led to a range of serious problems. Human beings not only expose them to extinction by overusing resources such as water, soil, forests, pastures and natural resources, but also threaten the earth by creating pollution from industrial activities, chemical fertilizers and pesticides (Reganold, 1992). For this reason, it is stated that intercropping practices in sustainable agriculture attract attention as an alternative and more ecologically compatible approach. Intercropping involves growing two or more crops simultaneously or sequentially within the same field, allowing for complementary interactions and increased resource use efficiency (Gomez and Gomez, 1983). This practice offers many benefits in terms of improving soil fertility, pest and disease management, weed control, conservation of biological diversity, water use efficiency, and overall sustainability. By bringing different plants together, intercropping enhances biological interactions and reduces dependence on external resources such as synthetic fertilizers and agricultural chemicals, thus minimizing the environmental impact of agriculture.

Intercropping Methods

Intercropping methods can be categorized into different groups based on the growth habit, growth stages, and interactions of plant species (Vandermeer, 1992; Ofori and Stern, 1987). Three commonly used intercropping groups:

1. Mixed Intercropping: In this method, the same plant species or different plant species are grown together in the same row. By reducing the spacing between plants, maximum land utilization is achieved, and the plants support each other. For example, maize plants can be intercropped with bean plants in the same row. Maize plants provide height and serve as support for bean plants, facilitating their growth. Mixed intercropping aims to maximize land utilization by reducing the spacing between plants. In this way, the field gets the most out of it by reducing gaps in the field and planting so that the plants support each other.

2. Strip Intercropping: This method allows for growing different plant species in separate rows on the same field. Instead of planting in the same row, the plants are grown in rows with gaps between them. Leaving gaps between rows provides enough space for the plants' growth and development. Strip intercropping enables more efficient use of field area and resources. The gaps between plant species allow for better spreading of root systems and leaves, enabling the plants to receive more sunlight, water, and nutrients. For example, maize plants can be intercropped with pumpkin plants in different rows. Maize plants provide height, while pumpkin plants spread in the gaps between rows, creating ground cover and suppressing weeds.

3. Temporal Intercropping: In this method, different plant species are planted at different times within the same field. This allows for a continuous crop throughout the season in the same area. For instance, a cool-season cereal crop can be planted first in a field, followed by another crop such as maize, soybean, or cotton as the second crop. This way, the field provides a continuous yield throughout the season.

Intercropping methods can vary depending on field conditions, plant species, and farmer preferences. There can be overlap between these groups, and in some cases, multiple intercropping methods can be used together.

Advantages of Intercropping Systems

The practice of cultivating plants together, known as intercropping or mixed cropping, offers several advantages. Some advantages of growing intercropping:

1. Soil fertility: Growing different plant species in the same area can enhance soil fertility (Eskandari, 2011; Mobasser et al., 2014). While one plant may deplete specific nutrients from the soil, others may have different nutritional requirements. In this way, the risk of one-sided nutrient depletion of the soil is reduced and the soil becomes more nutritionally balanced.

2. Pest and disease control: Intercropping is one of the integrated pest management strategies used to reduce the population density of pests in cultivated plants (Theunissen, 1997). Pests and diseases often focus on a single plant species, but intercropping with different plant species can hinder their spread (Danso et al., 1987). Additionally, certain plant species can act as natural pesticides or insect repellents, either deterring or attracting pests (Fujita et al., 1992).

3. Weed control: Weeds are known to negatively impact crop yields through competition or allelopathy. Intercropping is more effective in suppressing weeds compared to sole cropping (Girjesh and Patil, 1991).

4. Preservation of natural balance: Different plants in polyculture can help maintain a natural balance. For example, some plants can repel pests, while others can improve soil quality or attract natural enemies of pests. This reduces the need for chemical interventions and supports natural biological balance.

5. Increased productivity: One of the primary reasons for intercropping is to enhance overall yield (Caballero and Goicoechea, 1995).

Plant diversity enables more efficient utilization of soil resources and can lead to increased crop production. In addition, the different growth rates of plants and their ability to adapt to the seasons allow more effective use of the field or garden area.

6. Market opportunities: The higher returns derived from intercropping practices explain its suitability for commercial adoption (Yıldırım and Güvenç, 2005; Mahant et al., 2012). Growing different plant varieties together allows farmers to offer a range of products and expand market opportunities. It enables the cultivation of various crops tailored to market demands and consumer preferences, thereby diversifying farmers' income.

7. Water and energy conservation: Intercropping helps optimize water and energy usage. For instance, taller plants can provide shade and reduce water evaporation for shorter plants. It can also help plants use water and nutrients more effectively, as the root systems of plants spread to different depths.

8. Soil erosion prevention: Intercropping different plant species can reduce soil erosion. Plant roots help anchor the soil, thereby reducing the risk of erosion. Polyculture can play a significant role in combating wind and water erosion, particularly.

9. Biodiversity: Polyculture enhances biodiversity. Different plant species attract various animal and insect species, supporting the diversity of natural life within the ecosystem.

10. Sustainability: Intercropping contributes to agricultural sustainability. Reduced use of chemical fertilizers and pesticides leads to less soil and water pollution, causing less harm to natural ecosystems. Also, growing a variety of plants can help maintain ecosystems more resilient and long-term.

All these advantages demonstrate that intercropping makes agricultural production more sustainable, efficient, and diverse.

Conclusion

Intercropping systems are an important strategy for agricultural sustainability and productivity. By growing different plant species together, diversity and ecosystem health in agricultural areas are enhanced. Plant diversity allows for more efficient use of soil resources, resulting in increased soil fertility. Additionally, the spread of pests and diseases is prevented, reducing the need for chemical control methods. Intercropping reduces soil erosion, enhances water retention capacity, and promotes biodiversity. Furthermore, cultivating different crops in the same agricultural area offers marketing opportunities and diversifies farmers' income. Intercropping systems are a significant step towards achieving sustainability, environmental protection, and food security goals in the agricultural sector. However, careful consideration of factors such as plant selection, combination, and cultivation techniques are necessary. Selecting plant combinations that are suitable for the region, soil structure, and climatic conditions is crucial for the success of this system.

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CHAPTER 2

PROTECTIVE ROLE of SILIBININ on HEPATOTOXICITY of NICKEL SULFATE in RAT

Prof. Dr. Füsun TEMAMOĞULLARI [1]
Prof. Dr. Ahmet ATEŞŞAHIN [2]
Assoc. Prof. Dr. Nihat YUMUŞAK [3]
Assist. Prof. Dr. Mustafa Selim DOĞRU [4]
Res. Assist. Zozan GARİP [5]
Lecturer Dilek ARSLAN ATEŞŞAHİN [6]

---

[1] Harran Üniversitesi, Veteriner Fakültesi, Farmakoloji ve Toksikoloji Anabilim Dalı Şanlıurfa, Türkiye. farmafto@gmail.com, Orcid ID: 0000-0001-7738-1145

[2] Fırat Üniversitesi, Veteriner Fakültesi, Farmakoloji ve Toksikoloji Anabilim Dalı Elazığ, Türkiye. aatessahin@firat.edu.tr, Orcid ID: 0000-0002-1004-2146

[3] Harran Üniversitesi, Veteriner Fakültesi, Patoloji Anabilim Dalı Şanlıurfa, Türkiye. nihatyumusak@harran.edu.tr, Orcid ID: 0000-0002-9299-2902

[4] Sağlık Bilimleri Üniversitesi, Hamidiye Sağlık Hizmetleri Meslek Yüksekokulu, Eczane Hizmetleri Bölümü, İstanbul Türkiye. dr.mselimdogru@gamil.com, Orcid ID: 0000-0002-7301-4038

[5] Harran Üniversitesi, Veteriner Fakültesi, Farmakoloji ve Toksikoloji Anabilim Dalı Şanlıurfa, Türkiye. garipzozan@gmail.com, Orcid ID: 0000-0002-8971-7918

[6] Fırat Üniversitesi, Veteriner Fakültesi, Baskil Meslek Yüksekokulu Elazığ, Türkiye. datessahin@firat.edu.tr, Orcid ID: 0000-0002-1528-9367

INTRODUCTION

Nickel that is naturally found in the environment has a wide field of usage in modern industries. Nickel is an environmental pollutant that accumulates in kidneys, lungs, liver, heart, and testicles. The toxicity of nickel depends on the solubility of nickel compounds and exposure route [1]. Nickel has carcinogenic, teratogenic, immunotoxic, and genotoxic effects [2, 3]. Nickel nanoparticles have toxic effects on apoptosis, DNA damage, lipid peroxidation, liver, kidney, nerve cells, and reproductive system [4, 5]. They inhibit the activity of various enzymes (aspartate aminotransferase, alkaline phosphatase, ATPase, etc.) and thereby they affect many physiological events mediated by these enzymes [6]. It has been reported that nickel compounds cause hepatic degeneration and oxidative stress [1, 2, 7].

The efficacy of several antioxidant substances (such as zinc, L-ascorbic acid, moringa tea, naringin, selenium, and ginger) against oxidative damage caused by nickel in the liver has been investigated [2, 7–11]. Milk thistle plant (*Silybum marianum*) which is commonly found in Turkey includes several flavonoids such as primarily silibinin (SIL) which is a pharmacologically active ingredient, silychristin, silydianin, isosilbin, taxifolin, dehydrosilbin, and quercetin[12]. SIL is a valuable pharmacological substance because of its antioxidant activity, low unexpected effects, and safety [13]. The protective, canceroprotective, anticarcinogenic, and hypocholesterolemic effects of SIL have been reported in the prevention and treatment of liver diseases, acute mushroom poisoning, acute and chronic hepatitis, treatment of alcohol-induced or toxin-induced intoxication, and against hepatotoxic effects of many substances [13, 14]. It has been identified that many nickel compounds cause hepatic apoptosis [15]. In various studies, Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay and immunohistochemical staining method were used for assignment of apoptosis [16–18]. The appropriate caspase assay kit specific for mouse and human species (such as Caspase-3, Caspase-8, Caspase-9) is used to control cell apoptosis and inflammation [19]. TUNEL test is applied to identify DNA breaks [20].

The aim of the present study is to examine histopathologically and immunohistochemically the protective effect of SIL against nickel sulfate-induced hepatotoxicity in rats by TUNEL assay.

MATERIALS and METHODS

Ethical Statement

Approval from the Dollvet Inc. animal experiments local ethics committee (Approval no: Dolvet -HADYEK 2016/10 numbered) was obtained in order to conduct the study. The experiment protocol was approved by the Local Ethics Committee.

Experimental animals

Twenty-four healthy adult male Sprague Dawley rats, which were aged 6 weeks and had a weight of 200± 220 g, were used in the study and the experimental research unit of Fırat University supplied these animals. They were fed with a diet and water ad libitum in a cycle of 12 h light and 12 h dark at 23 ± 2ºC and 60–65% humidity. Their diet contained protein (22.21%), fat (3,32%), fiber (3.12%), carbohydrate (68%), vitamins, and minerals.

Experimental design

The rats were separated into four groups; Group 1, i.e. control group, (intraperitoneal injection of 1 ml isotonic saline solution, n=6), Group 2 (only Nickel sulfate (Acros Organics Nickel sulfate heptahydrate, Code:270552500, Lot: A0371683) at a dose of 5 mg/kg/day [21] n=6), Group 3 (only daily SIL at a dose of 150 mg/kg/day [22] n=6), and Group 4 (SIL at a dose of 150 mg/kg/day + nickel sulfate at a dose of 5 mg/kg/day n=6). Nickel sulfate was diluted with 1 ml isotonic saline solution throughout the study and injected intraperitoneally and SIL was suspended in a 0.3% carboxymethyl cellulose (CMC) (Sigma Chemical) solution and given orally [23, 24]. No death was observed in group 4 for 30-days application**.** After sacrificing the rats through cervical dislocation on the thirty-first day, we gathered liver tissue samples and frozen and stored them at 20ºC.

Histological procedures

For histopathological examinations, the tissue samples were fixed in buffered neutral 10% formaldehyde and embedded in paraffin blocks.

Afterwards, 4-mm thick sections were cut and stained with Hematoxylin–Eosin (HE) (Histostain Plus Kit; Zymed). Tissues were analyzed by using Olympus BX53 microscope (equipped with Olympus Cell B software) for image acquisition and analysis. Histopathological findings were assessed semiquantitatively as 0: none, 1: mild, 2: moderate, and 3: severe. The paraffin-embedded tissue blocks were cut in 4-µm sections on poly-l-lysine coated glass slides within the scope of the immunohistochemical examinations. All the tissues were examined using Streptavidin-Biotin-Peroxidase Complex method. Once routine deparaffinization and rehydration were completed, they were stained with the streptavidin-biotin–peroxidase complex (ABC) technique. Immunohistochemical staining was performed based on the method indicated by Yumusak et al. [20]. All tissues were marked with caspase3 (1: 200, Invitrogen), caspase8 (1:50, Abcam), and caspase9 (1: 100, Abcam) primary antibodies. The diaminobenzidine (DAB, Dako) was used as the chromogen. Cytoplasmic staining was rated according to brown staining presence as 0-negative, 1-weak, 2-intermediate, or 3-strong [25]. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay was applied according to the catalog procedure (In Situ Cell Death Detection Kit, POD; Roche, Germany) to detect apoptosis-related DNA breaks in liver cells. All procedures were carried out based on the method indicated by Yumusak et al. [20]. In final evaluation, staining indices were scored as 0-negative, <1% positive; 1-weak, 1–25%; 2-intermediate, >25–75%; or 3 – strong, >75% based on the rate of the stained nuclei.

Statistical Analysis

SPSS, Version 16.0 (SPSS Inc., Chicago, USA) was used for statistical analyses. Quantitative data related to tissue pathology were analyzed using Chi-square. When analysis of variance showed a difference between groups, Duncan test was used to determine the group causing the difference. In quantitative data, peak value, as a central tendency measure of variance in case of more than one peak value, was used for groups.

RESULTS

Pathologic results

The macroscopic examination of organs and tissues of the rats that underwent necropsy according to the routine necropsy technique revealed that liver tissues were severely hyperemic and partially pale yellowish-white necrotic fields were intensely observed in Group 2. It was noticed that similar lesions were less severe in Group 4 compared to Group 2. On the other hand, no macroscopic finding was determined in Group 1 and Group 3.

Microscopic results

In histopathological examination, vacuolization of hepatocyte cytoplasms was statistically significantly higher in Group 2 than other groups ($P<0.05$). Similarly, sinusoidal dilatation, degeneration, and necrosis in liver sections, and vesicles and anisokaryosis-like lesions in nuclei were statistically significantly more severely in rats in group 2 than those in Group 4 ($P<0.05$) (*Table 1, Fig. 1-a,b,c,d*). Immunohistochemically; cytoplasmic and nuclear apoptosis with varying degrees were detected in the other groups except Group 1 and Group 3 (*Figure. 2-b, d*). Caspase and TUNEL labellings were concentrated in the centrilobular fields. Hepatocytes around vena centralis were severely immunopositive, whereas perilobular fields were less affected. Additionally, all lobules were TUNEL positive from place to place. Many fields of liver tissue were immunohistochemically severely positive in Caspase-3, Caspase -8, Caspase -9, and TUNEL assay in Group 2 compared to the other groups ($P<0.05$). The rate of apoptosis was significantly higher for each three antibodies and TUNEL assay in Group 2 compared to Group 4 ($P<0.05$). No apoptosis was identified in the control group and Group 3 (*Fig. 2*).

DISCUSSION

Nickel is a substance that has hepatotoxic effect. It is known that nickel leads to tissue damage in the liver [26]. In their study, Pari and Amudha [11] detected impaired liver functions, histopathologically inflammatory cell infiltration, local necrosis, and giant cell formation in the group that was administered nickel. Also in the present study, higher hyperemia, severe necrosis, and vacuolization were identified in the livers of Group 2 exposed to nickel sulfate for 30 days compared to the other groups (*Figure. 1-b*). Das et al.

[9] stated that nickel sulfate led to lipid peroxidation and hepatocellular degeneration in the liver. In this study, a high degree of hepatic degeneration was determined in Group 2. In the present study, hepatic degeneration decreased in Group 4. Silibinin has been used as hepatoprotective against several poisonings (paracetamol, amitriptyline, erythromycin estolate, carbon tetrachloride, acetaminophen, ethanol, galactosamine, nortriptyline)[27]. In addition, many studies have reported that SIL acts as a free radical scavenger, increases cell membrane stability against hepatotoxicity and it has an antioxidant characteristic [28–31]. Pietrangelo et al.[32] identified the protective effect of silibinin against hepatotoxic effects of chronic high dose iron in rats. They stated that this protective effect might have been associated with the antioxidant effect of SIL. Similarly, Sözen et al. [13] reported that silibinin exhibited a tissue protective effect (cytoprotective effect) against hepatotoxicity of itraconazole by declining oxidative stress. It was determined in the same study that silibinin significantly reduced parenchymal hepatocyte apoptosis in the silibinin groups. It was determined also in the present study that apoptosis was less intense in Group 4 than Group 2 and SIL had a protective effect against hepatotoxic effect of nickel sulfate. In addition, SIL exhibited a protective effect against the side effects of nickel sulfate in the hepatic tissues belonging to Group 4 according to the findings of histopathological Caspase-3, -8, -9, and TUNEL assay. In their study, Raskovic et al. [33] reported that silymarin rich in silibinin showed a protective effect in the liver against the doxorubicin-induced hepatotoxicity in rats.

Nickel damages nuclear proteins by increasing the level of endogenous cellular hydrogen peroxide and decreasing the activities of the enzymes required for DNA replication, transcription, recombination, and repair [1]. Pari and Prasath [34] detected in their study that nickel caused an elevation in liver enzyme levels and impairment of cellular viability and integrity due to loss of hepatic membrane integrity. It was also accepted that the most important cause of nickel-induced liver damage was the intense accumulation of nickel in the liver [35]. Concepcion Navarro et al. [36] revealed that antioxidants were depleted during the detoxification of the toxic substances and they caused cellular damages in the liver. Numerous studies have reported that nickel induces liver damage through oxidative stress [4, 37]. Chen et al. [38] determined that free radicals led to tissue damages by causing lipid peroxidation in the cell membranes. In the present study, it is also thought that liver damage might have been caused by degenerations due to nickel accumulation in the liver and oxidative stress. In their study, Karagül et al. [39] suggested the curative effect of Silymarin against sodium fluoride-induced oxidative stress and degenerative changes in the liver in rats. Baeeri et al. [28] determined in their study that silibinin acted as an anti-aging because of its antioxidant effect in the embryonic fibroblast cells in rats. It was histopathologically detected in the present study that silibinin decreased hepatic degeneration against nickel intoxication.

The caspase-3, -8, and -9 activities and TUNEL assay are used for the detection of liver apoptosis. The apoptosis was identified using the activity of related caspase-3, -8, and -9 activities and TUNEL assay in the rats to which copper sulfate was administered for different durations (14 and 28 days) [40]. In another study, Prabu and Muthumani [41] revealed that caspase-3 immunoreactivity increased compared to the control group in the rats exposed to arsenic. In their study, Massányi et al. [17], analyzed the changes induced by nickel in the male mice testicles with TUNEL assay and identified that localized apoptosis was more intense in the nickel administered group. Likewise, in another study conducted using heavy metal, caspase-3 and TUNEL assays were used for identification of apoptosis induced by cadmium in rat testes and a significant increase was determined in the rate of apoptosis in the cadmium administered group[16]. Yalçin and Pekmez [18] reported in their study that acrylamide significantly increased caspase-3 immunoreactivity against acrylamide-induced hepatotoxicity in rats in the acrylamide group compared to the control group. Also in the present study, various degrees of apoptosis were determined in the other groups except for the control group and Group SIL in the analysis carried out in liver tissues by immunohistochemical method and TUNEL assay. Particularly, the results obtained in the Group 2 for Caspase-3, -8, -9 and TUNEL assays were significantly more severe compared to the other groups. On the other hand, Caspase-3, -8, -9 and TUNEL activities were lower in Group 4. It was thought that lower Caspase-3, -8, -9 and TUNEL activities in Group 4 might have been caused by the antioxidant effect of SIL.

As a conclusion, it was determined that SIL can be used as hepatoprotective in the rats exposed to nickel sulfate.

Table 1. Histopathological scoring of liver tissue in rats

Tissue	Variables	Group 1	Group 2	Group 3	Group 4
Liver	Hepatic degeneration	-	+++	-	++
	Hepatic necrosis	-	+++	-	++
	Nuclear vesiculation	-	+++	-	++
	Hepatocyte anisokaryosis	-	+++	-	++

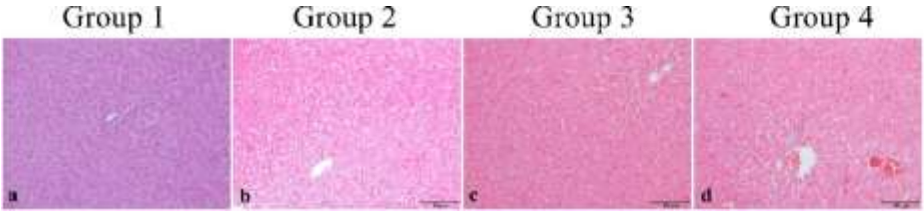

Figure 1. Histopathological findings of liver tissue

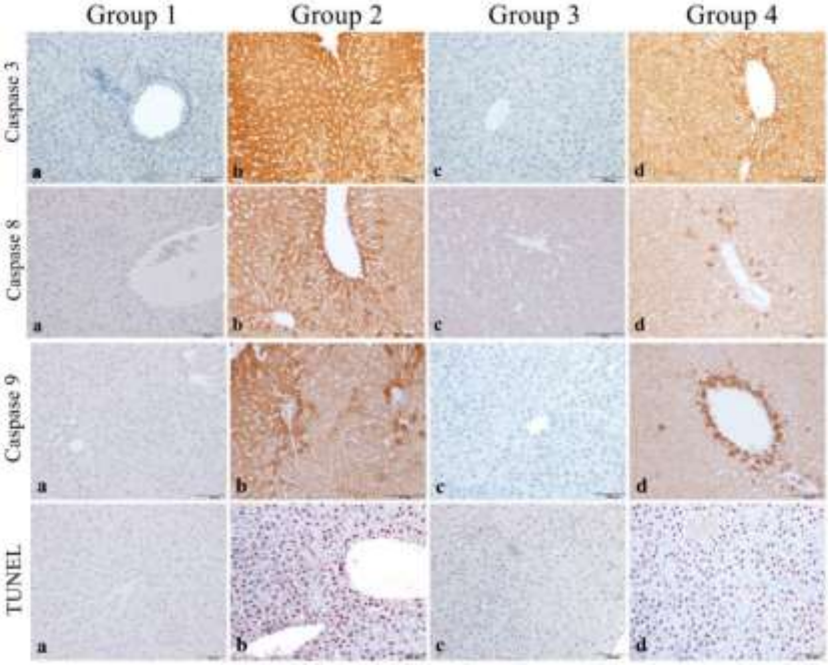

Figure 2. Immunohistochemical findings in liver tissue

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CHAPTER 3

HEAT STRESS IN WHEAT (*Triticum aestivum* L.): AN OVERVIEW

Assist. Prof. Dr. Rıdvan UÇAR[1]

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[1] Pamukkale University, Faculty of Agriculture, Department of Field Crops, Denizli
Orcid ID: 0000-0001-6365-7200, E-mail: rucar@pau.edu.tr

1. Introduction

Wheat may experiences heat stress at many growth stages. Flowering and reproductive stages are especially vulnerable because flowering often takes place in cooler environmental conditions. A wheat plant's biochemical, physiological, and morpho-anatomical behaviour can vary in response to high temperatures. To develop unique crop varieties that can deal with shifting climatic conditions, it is imperative to have a thorough understanding of the physiological, biochemical, and morphological impacts of heat stress. Manipulation of flowering time for adaptation through natural or genetic approaches may alleviate heat-stress damage.

More than 80% of the world's population utilises wheat (*Triticum aestivum* L.) as a primary source of protein and calories. Concern is growing across the globe regarding the impact of rising temperatures on wheat production due to recent global climate change. The main abiotic stresses limiting wheat yield are heat and drought. The crucial physiological and biochemical processes of the plant are halted by heat stress. Stress brought on by high temperatures decreases the quantity of grains, photosynthetic activity, chlorophyll content, and endosperm starch synthesis. The crop suffers significant oxidative damage when reactive oxygen species which have accumulated due to heat stress. In order to reduce the effects of heat stress, plants rapidly synthesise heat shock proteins. Heat tolerance is greatly influenced by a number of properties, including stay green, chlorophyll fluorescence, and canopy temperature. Knowledge of the physiological, biochemical, and morphological effects of heat stress is essential to developing novel varieties of crops that can withstand changing climatic conditions (Poudel & Poudel, 2020).

2. Physiological and molecular responses of wheat to heat stress

Wheat production is threatened by high temperature stress, which prevents photosynthesis (Coast et al., 2022). Wheat's carbon balance and growth are primarily determined by photosynthesis and respiration, both of which are susceptible to high temperatures. Wheat can adapt its respiration and photosynthesis to high temperatures, minimising the detrimental effects on growth. Future steps towards lowering heat-induced yield losses could involve improving these systems to better accommodate warmer climates. However, much remains to be learnt about such phenomena. There may be numerous ways to increase wheat's endurance to high temperatures depending on how photosynthesis and respiration react to the temperature. Extending our knowledge in this area could also lead to more immediate benefits, such as the enhancement of current crop models (Posch et al., 2019).

Wheat experiences heat stress at many growth stages, although flowering and reproductive stages are especially vulnerable because flower opening often takes place in cooler climates. Ovule and pollen sterility as well as anther dehiscence are all results of heat stress during meiosis. Temperatures greater than 30°C results with pollen abortion during the growth stage. Heat stress inhibits the transfer of resources to the growing grain at anthesis, resulting with small grains and lower yields. Due to decreased activity of the enzymes involved in grain biosynthesis, decreased flag leaf assimilatory efficiency, and poor stem reserve mobilisation, heat stress during grain growth shortens the grain-filling period and reduces starch and protein accumulation. The development of heat-tolerant wheat genotypes through screening, selection and breeding using genetic engineering, exogenous application of osmoprotectants and agronomic approaches has high priority (Ullah et al., 2022).

High temperatures wheat plant's grain filling period and inhibits the endosperm's capacity to synthesise starch. Post-anthesis heat stress reduces starch production and changes the composition of starch, whereas temperature beyond the optimum during flowering results in sterility in the seed. To deal with heat stress, wheat crops have developed suitable strategies such as escape, avoidance, and/or staying green. In addition, plants hasten the production of heat stress-related proteins such as heat shock proteins as their defense approach (Sharma et al., 2019).

Germination, seedling establishment, source-sink activity, leaf area, shoot growth, and root growth are among the morphological parameters impacted. Heat also has an impact on the physiological processes including leaf senescence, and the relationship between water and nutrients. Heat stress causes the production of reactive oxygen species at the cellular level, which disrupts the thylakoid, chloroplast, and plasma membrane membrane system. The formation of photoassimilates and their distribution are impacted by the photosystem's inactivation, decreased photosynthesis, and inactivation of rubisco. This ultimately affects anthesis, grain filling, size, number and maturity of wheat grains, which hamper crop productivity. The interplay of various systems comprising antioxidants and hormones plays a crucial role in imparting heat stress tolerance in wheat. Thus, implementation of various omics technologies could foster in-depth insights on heat stress effects, eventually devising heat stress mitigation strategies by conventional and modern breeding to develop heat-tolerant wheat varieties (Lal et al., 2021).

The majority of plant species have developed a strong signal transduction system that allows them to sense changes in the ambient temperature, transmit this information, and then modify their metabolism and cellular processes to prevent heat stress-related damage. The cell membrane/plasma membrane plays prominent roles as an interface system that perceives and translates the changes in environmental signals into intracellular responses. Thus, membrane lipid composition is a critical factor in heat stress tolerance or susceptibility in wheat (Abdelrahman et al., 2020). Moderate heat stress may provide protection against a subsequent severe high temperature stress in plants. However, the exact mechanisms of heat acclimation of wheat are still poorly understood (Janda et al., 2019).

3. Breeding wheat against heat stress

Using various genetic approaches we can develop heat tolerant wheat varieties in order to mitigate the effect of heat stress on wheat production. Currently different strategies have been adopted to develop thermotolerance in wheat. Development of thermotolerant wheat varieties is one of the major steps toward the improvement of wheat yield against heat stress. For this purpose, it is important to have a complete and clear concept of the morpho-physiological, biochemical and molecular mechanisms of heat tolerance in wheat. Better knowledge about heat tolerance through discussing the morphological, physiological, biochemical and molecular mechanisms of heat tolerance in wheat based on different parameters such as grain filling duration, grain yield, leaf senescence, canopy temperature depression, photosynthesis, chlorophyll content, membrane thermostability, translocation of photo-assimilates, starch synthesis, antioxidant response, protein synthesis and omics approaches are required (Sarkar et al., 2021).

Various physiological mechanisms have been identified as selection targets for heat tolerance; however, the complex nature of the trait and high genotype × temperature interaction limits the selection process (Ullah et al., 2021). While various traits linked to high-temperature tolerance have been identified, the combination of traits that optimize the heat tolerance of wheat has not been established in most environments. High temperature reduced yield, plant height, grain weight and days to anthesis and maturity, and increased the percentage of screenings and grain protein content. Genotypes that produced higher yield under heat stress had shorter days to flowering and maturity, higher NDVI during grain filling, greater chlorophyll content at the milk stage of grain fill, taller plants, greater grain weight and number, and lower screenings (Ullah et al., 2020).

It is known as "terminal heat stress" when a wheat crop is subjected to high temperatures during the blooming and grain-filling stages. To facilitate the selection of genotypes for improved performance under severe heat stress, adaptive features should be characterised (Rehman et al., 2021). Developing resistant cultivars through the traditional breeding method, which entails selecting tolerant lines and then adding the tolerant characteristics in commercial types, is one strategy to reduce the damage caused by high temperatures (Fu et al., 2023). Understanding the plasticity of the response to heat stress that exists between wheat genotypes, especially in source–sink relationships at the reproductive and grain-filling stages, is critical for the selection of germplasm that can maintain high yields under heat stress. A broad understanding of metabolic dynamics and the relationships between metabolism and heat tolerance is required in order to achieve this goal (Abdelrahman et al., 2020).

Breeding for stable yield across both good and bad seasons while maintaining high yield under optimal conditions is a high priority for most wheat breeding programs and has been a focus of research activities. Multiple strategies have been explored to enhance the heat and drought tolerance of wheat including extensive genetic analysis and modify the expression of genes involved in stress responses, targeting specific physiological traits and direct selection under a range of stress scenarios. These approaches have been combined with improvements in phenotyping, the development of genetic and genomic resources, and extended screening and analysis techniques. The results have greatly expanded our knowledge and understanding of the factors that influence yield under stress, but not all have delivered the hoped-for progress (Langridge & Reynolds, 2021).

The development of thermotolerant wheat cultivars using conventional or molecular breeding and transgenic approaches is promising. Over the last decade, different omics approaches have revolutionized the way plant breeders and biotechnologists investigate underlying stress tolerance mechanisms and cellular homeostasis. Therefore, developing genomics, transcriptomics, proteomics, and metabolomics data sets and a deeper understanding of heat stress tolerance mechanisms of different wheat cultivars are needed (Yadav et al., 2022). Manipulation of flowering time for adaptation through natural or genetic approaches may combat heat-stress damage that occurs at the reproductive stages in production conditions (Bellundagi et al., 2022).

Wheat is challenged by increasing temperatures, however its polyploid nature, the incompleteness of its genome sequences and annotation, the lack of comprehensive heat stress-responsive transcriptomes and the unexplored heat sensing and signaling of wheat hinder our full understanding of its adaptations to heat stress. The recently released genome sequences of wheat, as well as emerging single-molecular sequencing technologies, provide an opportunity to thoroughly investigate the molecular mechanisms of the wheat response to heat stress (Wang et al., 2019).

It is important to compare wheat responses to heat at seedling and reproductive stages, to explore the potential relationship between the performances at different growing stages and the possibility of early selection to accelerate heat tolerance breeding. It was found that root lengths at seedling stage were severely reduced by heat stress with significant variations among wheat genotypes. Heat-tolerant genotypes at seedling stage showed less root length decrease than susceptible ones. Wheat genotypes tolerant at seedling stage showed higher yield at adult stage after heat treatment. A significant positive relationship was found between heat tolerance at seedling stage and at adult stage, suggesting a similar tolerant/susceptible mechanism at different plant growth stages and the possibility of early selection at seedling stage for breeding heat tolerance (Lu et al., 2022).

4. Agronomic management of heat stress in wheat

Wheat can be grown successfully in a warmer environment through manipulating some agronomic management practices (Ortiz et al. 2008). Adoption of various agronomic practices like 1) water conserve techniques 2) the appropriate amount and methods of fertilization 3) maintaining proper time and methods of sowing, and 4) the application of exogenous protectants can effectively alleviate the adverse impact of heat stress in wheat (Singh et al. 2011b).

According to Hakim et al. (2012), late-sown wheat varieties typically experience severe temperature stress, which shortens the heading and maturity periods and eventually affects grain production and quality. As a result, choosing genotypes with early maturity and a relatively long time to heading is advised for the breeding of high yielding wheat cultivars adapted to semiarid conditions (Al-Karaki 2012). Hence, the early planting and the genotypes with early maturity and a relatively long time to heading are advocated to evade terminal heat stress and accelerate grain filling (Khichar and Niwas 2007). In order to get the optimum plant growth and yield of wheat in a heat-stressed climate, keeping the proper planting time is one of the most crucial agronomic practises (Kajla et al. 2015). Planting of wheat in conventional tillage with straw mulch increased water holding capacity, organic carbon, and total nitrogen in soil and improve tillering capacity resulting to mitigate the high temperature-induced reduction of grain weight at the late grain filling stage (Tang et al. 2013).

When the day and night temperatures are 24 and 17°C, respectively, the application of nitrogen, phosphorus, and potassium during the post-anthesis period enriches grain proteins. However, effects are eliminated for higher day and night temperatures. Nutrient foliar spraying is particularly efficient at reducing the negative effects of heat stress on wheat. Alternative methods to improve wheat's resistance to heat include spraying it on the leaves after anthesis with potassium orthophosphate (KH2PO4). The delay of heat stress-induced leaf senescence and increased grain production are both effects of potassium orthophosphate (Dias and Lidon, 2010). The benefits of NO3 in delaying the production of abscisic acid and enhancing cytokinin activity, as well as K+-induced increases in photosynthetic activity and assimilate accumulation, are well known for increasing grain yield in heat-stressed environments (Akter & Rafiqul Islam, 2017). Plants are encouraged to tolerate heat through the exogenous administration of calcium (Waraich et al. 2011). Magnesium (Mg) supplementation was found to be a successful nutritional tactic for reducing heat stress-related losses in wheat production. Mengutay et al. (2013) found that wheat plants with Mg lack were vulnerable to heat stress and that Mg in the form of MgSO4 was sufficient.Heat stress generally increases Zn concentration in grain mostly due to remobilization from the shoot (Dias and Lidon, 2009). Therefore, Zn has also been proven to be effective in improving heat tolerance in wheat.

A different approach to increasing heat tolerance is currently being studied by Raijmakers et al. (2009), who involved biological control agents like fungi and bacteria. The growth of wheat plants under heat stress is found to be compatible with and to benefit from the presence of plant growth-promoting rhizobacteria (Nain et al., 2010). Wheat's ability to withstand heat was improved by seed treatment with rhizobacteria and foliar application of several organic and inorganic chemicals (Yang et al., 2009). Wheat's ability to withstand heat was also greatly enhanced by rhizobacteria seed treatment (Anderson and Habiger 2012).

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CHAPTER 4

PHYTOBIOTICS IN POULTRY NUTRITION: USE AND IMPORTANCE

Assist. Prof. Dr. Besime DOĞAN DAŞ[1]

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[1] Harran University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disease Şanlıurfa, Türkiye. bdas@harran.edu.tr, Orcid ID: 0000-0003-2163-2632

INTRODUCTION

Antibiotics are chemical substances that are produced by various microorganisms such as fungi, bacteria, and actinomycetes and are also prepared synthetically, that stop the development of pathogenic microorganisms or kill them. However, continuous and low-dose antibiotic use has led to bacterial resistance and the development of new bacterial strains in animals and humans consuming these animal products (Aarestrup et al., 2000). For this reason, the use of antibiotics as feed additives has been banned in many countries (Cervantes, 2006). With the prohibition of antibiotics in the poultry industry, the search for natural growth factors has come to the fore.

Phytobiotics are completely of vegetable origin and are obtained by extraction from phytogenic (aromatic) plants. “Phytobiotics” or “phytogenic feed additives” is a term used for plant extracts obtained from aromatic and spicy plants that have aromatic properties as well as functional properties when added to animal feeds. Phytobiotics are compounds of plant origin and can improve animal productivity. The leaves, flowers, seeds, roots, woody structures of plants and essential oils and extracts of these plant parts are included in the concept of phytobiotics (Jacela et al., 2010). The active ingredient content of phytogenic feed additives can be very variable depending on the plant used and the botanical part of the plant (seeds, leaf, root or bark), harvest time and geographical origin. Processing techniques (such as cold pressing/distillation, steam distillation, extraction with non-aqueous solvents) also alter the active ingredients and related compounds in the final product (Windisch et al., 2008). Phytobiotics can contain many different bioactive elements such as alkaloids, flavonoids, glycosides, mucilage, saponins, tannins, phenolics, polyphenols, terpenoids, polypeptide, thymol, cineol, linalool, anethole, allicin, capsaicin, allyl isothiocyanate and piperine (Upadhaya and and Kimya, 2017). It is known that phenolic compounds (caffeic cinnamic, ferulic acid, gallic acid, oleuropin, thymol, eugenol), organic acids (benzoic, sorbic, citric, acetic acids), essential oils (allyl, isothiocyanate, allicin) obtained from plants have antimicrobial effects. (Yel, 2011). The mechanism of action of phenols is related to the increase in cell wall permeability by denaturing the proteins in the bacterial cell wall. As a result, the permeability of the cell wall is impaired and the bacteria die when the intracellular fluid escapes from the cell. Since antibiotics such as penicillin and streptomycin affect the chromosomal structure of bacteria, resistance can occur. Phenol compounds cause the destruction of bacteria by directly affecting the cell wall (Anonim, 2004).

COMMON CHARACTERISTICS AND ACTION MECHANISMS OF PLANT EXTRACTS

Plant extracts or phytogenic feed additives (often also called phytobiotics or botonics) can generally be defined as compounds derived from plants that are added to the rations of animals, improve the characteristics of the feed, improve the performance of the animals as well as the quality of the food obtained from these animals. It is known that phenolic compounds (caffeic cinnamic, ferulic acid, gallic acid, oleuropin, thymol, eugenol), organic acids (benzoic, sorbic, citric, acetic acids), essential oils (allyl, isothiocyanate, allicin) obtained from plants have antimicrobial effects. Numerous in vitro studies have been conducted on the antimicrobial effects of plant extracts, and it has been determined that these substances have antimicrobial properties against many pathogens (Adam et al., 1998; Burt, 2004; Dorman and Deans, 2000; Hammer et al., 1999; Özer et al., 2007; Si et al., 2006; Smith-Palmer et al., 1998). The compounds in their structures that show these effects are generally the parts that show the antioxidative effect, and phenolic compounds, which are basically active elements, are considered to show this effect.

Possible benefits of using herbal extracts in poultry feed are more weight gain, higher egg production and better feed utilization, killing pathogenic microorganisms in the digestive system from the mouth, increase flavor in feed, increase secretion of digestive juices, increasing the digestibility of feeds by increasing the efficiency of digestive enzymes, strengthening the immune system, providing low-cholesterol animal products, producing better quality and lean meat by stimulating protein synthesis, and creating a cleaner and healthier environment by binding ammonia (Gill, 1999).

Plant extracts and essential oils show antioxidant properties in the body as well as antimicrobial properties. Studies have shown that the shelf life of animal products is extended if essential oils obtained from many herbs and spices are added to the ration (Botsoglou et al., 2004).

It is claimed that phytogenic feed additives improve the taste and palatability of feeds and accordingly increase animal performance. However, there are very few studies showing this specific effect. Many spices and herbs and their extracts are used as drugs due to their beneficial effects in the digestive tract (for example, relief of flatulence, spasmolytic and laxative effects) (Chrubasik et al., 2005). The active components and mechanism of action of some plant extracts are presented in Table 1.

Tablo 1. Active components and the mechanisms of action of some plant extracts

Plant common name	**Plant Latin name**	**Major active compound**	**Mechanism of action**
Thyme	*Tymus vulgaris*	Thymol	Stimulation of digestion, antiseptic, antioxidant
Sage	*Salvia apiana*	Cineole	Stimulation of digestion, antiseptic
Daphne	*Laurus nobilis*	Cineole	Stimulation of digestion
Rosemary	*Aniba rosaeodara*	Cineole	Stimulation of digestion, antiseptic, antioxidant
Clove	*Syzgium aromaticum*	Eugonol	Stimulation of appetite and digestion, antiseptic
Anise	*Illicum verum*	Anethole	Stimulation of digestion, stimulation of milk yield
Ginger	*Zingiber officinale*	Zingerole	Stimulation of gastric secretion
Coriander	*Coriandum sativum L.*	Unalol	Stimulation of digestion
Mint	*Mentha piperita*	Menthol	Appetite enhancer, digestive stimulant, antiseptic
Garlic	*Allium sativum*	Allicin	Stimulation of digestion, antiseptic

Essential oils are mixtures of terpenoids, low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, and lactones. Essential oils are defined as natural products formed in plants, volatile with water vapor, liquid at room temperature, obtainable by extraction or distillation method, colorless or light yellow, strong odor specific to the plant, and consisting of many components, with a burning flavor. It is also known as an essential oil, and spirit (Sevinç and Merdun, 1995). Alcohols, esters, terpenes, aldehydes, and coumarins in the structure of essential oils give the plant many properties such as antiseptic, antispasmodic, analgesic, antiviral, bactericidal, antidepressant, and blood pressure regulator. Although it is not yet completely known how essential oils help with the digestion of nutrients, it is known by many scientists that essential oils have a stimulating effect on the digestive system. 1/3 of nearly 300 plant families grown in nature contain essential oil. Of the approximately 3000 known essential oils, about 300 are of commercial importance, and some of them or some of the components they contain are used in pharmacology, agricultural applications, preservatives, and flavorings in the food industry, alcoholic beverages, animal nutrition, sanitation, cosmetics and perfumery products, and natural therapeutic folk medicine (Hajhashemi et al., 2003; Perry et al., 2003; Silva et al., 2003).

The most researched aspect of essential oils relates to their antimicrobial activity. Since these oils are complex mixtures containing different components, their degree of effectiveness varies depending on the type and amount of active ingredients they contain (Toroğlu and Çenet, 2006). Essential oils have antibacterial effects on many microorganisms, including Gram (-) and Gram (+) bacteria. AlHowiriny (2003) extracted the essential oil of the Salvia lanigera plant and reported that this extract showed a very good inhibitory effect against *Bacillus subtilis*, and *Candida vaginalis* microorganisms, but *Escherichia coli* and *Pseudomonas aeruginosa* were resistant to this essential oil. It has also been suggested that there are antagonistic and synergistic interactions between the components of essential oils (Burt, 2004). Some plant extracts and active compounds have natural fungistatic and/or fungicidal effects against foodborne pathogenic molds.

The antioxidative properties of essential oils, which play a role in antifungal effects, are due to the phenolic hydroxyl groups in the structure of the components they contain (Cuvelier et al., 1996). The antioxidant effect of these oils varies according to the amount of active ingredients they contain, the type of solvent used in the extraction, and the extraction method (Vekiari et al., 1993).

THE IMPORTANCE OF PHYTOBIOTICS IN POULTRY NUTRITION

Recently, aromatic plants and extracts have attracted the attention of scientists who are in search of natural and safe substances that can be an alternative to antibiotics in broiler farming, and research has begun to determine the effects of these substances on the organism. Medicinal and aromatic plants and the active components of essential oils obtained from them have been reported to have antimicrobial, antioxidant, antilipidemic, antifungal, antiviral, digestive system stimulant, performance-enhancing, feed efficiency, and improving vitality effects (Adıyaman and Ayhan, 2010). Antimicrobial activities of essential oils occur by inhibiting/degrading certain activities (transport of electrons, ion density, phosphorylation, enzyme-dependent reactions) in the cell wall of the microorganism they are in contact with (Ultee et al., 1999; Dorman and Deans, 2000). In addition, essential oils show a high affinity for lipids in the bacterial cell wall (Jouany and Morgavi, 2007). However, the antimicrobial effect of essential oil depends on its chemical structure, namely the chemical compounds it contains. Therefore, the antimicrobial effect level of essential oil against different microorganisms will not be the same because the cell wall structures of the microorganisms are different. While researching alternative additives to antibiotics, some plant seeds or leaves were directly ground into the ration, and experiments were carried out. When black cumin seeds were added to the laying hen's rations at different levels (5, 10 and 15%), it was observed that egg weight and feed efficiency increased, egg cholesterol level and saturated fatty acids decreased (Yalçın et al., 2010). In a study, when 100, 200, and 400 mg kg-1 anise oil was added to broiler rations as an alternative to antibiotics (10 mg kg-1 avilamycin), the highest body weight and the best feed conversion ratio were obtained in the group given 400 mg kg-1 anise oil, and anise oil can be used as a natural growth factor (Çiftçi et al., 2005). Some studies with broilers (Mitsch et al., 2004; Jamroz et al., 2003; Jamroz et al., 2005) have shown that essential oils have antimicrobial effects against *E. coli* and *Clostridium perfringens* in vitro.

STUDIES ON PHYTOBIOTICS IN THE NUTRITION OF POULTRY

Bölükbaşı et al. (2011) investigated the effects of thyme (*Tymus vulgaris*) herb added to egg chicken diets on triglyceride and cholesterol levels in blood serum and egg yolk, and the number of *E. coli* in feces. In the study, 64 Lohmann LSL hybrid layer hens at 24 weeks of age were divided into four groups. The study was carried out with four replications for each group. The diets were prepared by adding thyme herb to the basal feed at a rate of 0, 0.1, 0.5, and 1%, respectively. In the study, it was determined that thyme added to the feed did not affect the egg yolk triglyceride and cholesterol ratio, but the addition of 0.1% thyme grass significantly reduced the blood serum triglyceride and cholesterol levels. They reported that the use of thyme at the level of 0.1 and 0.5% in laying hen diets significantly reduced the number of *E. coli* in feces.

Çiftçi et al. (2018), in their study, aimed to determine the effect of adding coriander oil to layer hen rations on performance, egg quality characteristics, egg yolk Thiobarbituric acid reactive substance (TBARS) values, and some serum parameters. They used 96 24-week-old Lohmann white laying hens, with 24 animals in each group, as 1 control and 3 experimental groups. The control group (K-0) was fed with commercial layer chicken feed, other groups were treated with coriander oil at basal feed at 0.1% (KY-1), 0.3% (KY-2), and 0.5% (KY-3) levels, respectively. They were fed with the rations formed by the addition of the diet for a total of 12 weeks, 2 weeks of which was a period of acclimatization to the trial rations. It was determined that the daily feed consumption, feed conversion rate, and damaged egg rate were not affected by the performance characteristics, but they increased the egg production of all groups and decreased the egg weight compared to the control. They found that treatments had no effect on egg quality criteria. Although coriander oil reduced the cholesterol value from serum blood parameters, other blood parameters were not affected by the treatments. No significant difference was observed between the groups in terms of egg yolk TBARS values during the storage period of the eggs (0, 7, 14, and 28 days).

In her study, Şehitoğlu (2019) determined the effect of clove oil supplementation at increasing levels (50, 100, and 150 ppm) in laying hen rations on performance, egg quality, some blood parameters, and egg yolk

TBARS (Thiobarbituric acid reactive substance) values. In the study, 96 Lohman white layer hybrid hens at 28 weeks of age were fed commercial layer chicken feed and basal feed with rations that were supplemented with 50 ppm, 100 ppm, and 150 ppm clove oil, respectively, for a total of 13 weeks, the first week of which was an acclimatization period. It was determined that increasing levels of clove oil added to the ration did not affect body weight. In the polynomial analysis, it was determined that other parameters (daily feed consumption, egg weight, damaged egg rate) did not change, except for egg production and feed conversion ratio. It was determined that depending on the level of increasing clove oil in the ration, the feed efficiency improved linearly, while the egg yield increased linearly. It was determined that egg quality parameters (egg weight, shape index, breaking strength, shell weight, shell thickness, yolk color, yolk index, white index, and Haugh unit values) were not affected by the treatment. It was determined that other serum parameters (triglyceride, glucose, aspartate aminotransferase, alanine aminotransferase, and calcium) were not affected by the addition of increasing levels of clove oil to the ration, except for cholesterol, albumin, alkaline phosphatase, and phosphorus. As a result, it was concluded that clove oil can be used at a level of 150 ppm in laying hen rations in order to extend the shelf life of eggs and reduce the amount of serum cholesterol.

Kırar et al. (2020) determined the effects of sumac added to the feed of Japanese quails at different levels on body weight gain (BWG), feed consumption (FC), feed conversion ratio (FCR), oxidative stress parameters, and meat quality. In the study, 120 mixed-sex Japanese quails 10 days old were randomly divided into 4 groups, one of which was the control group. They divided the animals in each group into 15 replicates in pairs. The rations were prepared as isocaloric and isonitrogenous by adding 0%, 1%, 2%, and 3% sumac powder to quail feeds, respectively. In the study, they determined that the addition of sumac had no effect on BWG, FC, and FCR. While the addition of sumac did not affect BWG, FC, and FCR, significant differences were observed between the groups in terms of back and thigh weights. They also determined that sumac did not differ between the groups in terms of breast meat pH and breast meat L*, a*, and b* values at the 1st and 24th hours. The highest total antioxidant status (TAS) value in the blood was determined in 3% sumac and the lowest in the control group. They found that the highest oxidative stress index (OSI) value was in the control group, and the lowest in the 3% sumac group.

Daş et al. (2020) determined the effect of peppermint oil added to quail rations on growth performance, meat quality, color, and blood oxidative stress properties. For this purpose, they used 40 Japanese quails at the age of 10 days and continued the experiment for 35 days. The research groups were divided into 4 groups, each of which had 10 quails individually. In the rations prepared for quails, no feed additives were used in the control group, while 0.1%, 0.2%, and 0.3% peppermint oil (NY) were added to the feeds of the other groups. There was no difference between the groups in terms of body weight (CA), daily live weight gain (GCAA), daily feed consumption (GYT), feed conversion ratio (FCR), carcass and slaughter characteristics, color, and pH throughout the experiment. However, they found that it significantly decreased the total oxidative status (TOS) and increased the total antioxidant status (TAS) in the blood. As a result, they stated that adding peppermint oil to quail rations did not change the fattening performance and carcass characteristics, but increased TAS values and decreased TOS values, so it would be beneficial to add 0.1% NY to the ration as a feed additive.

In her study, Saçıldı (2013) determined the effects of different levels of aged garlic extract added to broiler rations on performance and meat quality. In the study, 540 day-old broiler chicks (Ross 308) were randomly placed in a total of 36 compartments, consisting of 6 treatment groups with 6 replications, each with 15 animals of similar live weight. For 42 days, 5,10, and 15 ml/kg feed aged garlic extract, 10 ml/kg feed fresh garlic extract, and 200 ppm/kg feed vitamin E addition as a comparison group were fed to the control group with standard broiler chicken feeds. The body weight, live weight gain, feed consumption, and feed efficiency ratios of broilers were determined by weighing made at 0, 21, and 42 days. In addition, the chemical content of the meat, color, pH values, TBA level, and sensory tests of the meat was performed as meat quality in the experiment. It was reported that the addition of aged garlic extract to the rations significantly increased body weight, live weight gain, and feed consumption. He also concluded that it causes significant decreases in blood cholesterol and TBA levels and that adding aged garlic extract to the rations can extend the shelf life of meats by lowering blood cholesterol and TBA.

Şahin et al. (2020), in their study, determined the effects of herbal extract mixture added to broiler rations at different levels on performance carcass parameters and some visceral weights. In the study, a total of 192-day-old mixed-sex broiler chicks (Ross 308) were divided into four groups with 48 chicks each. A control and three experimental groups were formed in the experiment. In the experiment, the basal ration (without additives) was prepared in accordance with the starting and finishing periods. They added 1gr/kg, 2gr/kg, and 4gr/kg herbal extract mixtures to the diets of the experimental groups, respectively. The trial continued for 42 days. They reported that the herbal extract mixture (BEK) added to the rations at the end of the experiment did not create statistically significant differences in performance parameters (GCAA, GYT, YYO), slaughter parameters, and visceral weights. As a result, they concluded that the herbal extract mixture did not cause significant changes in the performance values, carcass, and visceral weights, but it would be beneficial to determine the doses of the optimum mixtures of the aforementioned additives and to examine the effects on animals at different doses in different periods.

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CHAPTER 5

GENETICS AND BREEDING OF DROUGHT TOLERANCE IN SUNFLOWER (*Helianthus annuus* L.)

Dr. Abdullah ÇİL[1]

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[1] Republic of Türkiye Ministry of Agriculture and Forestry, Eastern Mediterranean Agricultural Research Institute, Field Crops, Adana, Türkiye. acil70@hotmail.com, Orcid ID: 0000-0003-3482-6946

1. Introduction

The most detrimental environmental stress is considered to be drought, which is known to have the greatest negative impact on crop yields. Numerous drought indices have been developed in an attempt to identify, track, and describe drought events. Sunflower has a strong root system that can absorb water from deeper soils. Although, sunflower is a moderately drought tolerant crop, severe drought events cause reductions in the seed and oil yields. Different management techniques, such as conventional or biotechnological breeding for drought tolerance, exogenous administration of hormones and osmoprotectants, seed treatment, and soil nutrient management methods have been suggested. Finding the genes that truly affect drought tolerance among the vast number of genes is one of the most challenging tasks in sunflower genetic breeding.

The main factors restricting the development, productivity, and distribution of plants are environmental stressors. Arid or semi-arid regions constitute one-third of the earth surface. Poor weather and unfavourable soil conditions are among the main barriers to high yielding sunflower (*Helianthus annuus* L.) production (Li et al., 2021). The most detrimental environmental stress is considered to be drought which is known to have the greatest negative impact on crop yields. Plant growth and development are significantly hampered by drought, with significant decreases in crop growth rate and biomass accumulation. The main consequences of drought in plants are reduced rate of cell division and expansion, stem elongation and root proliferation, leaf size, and disturbed stomatal oscillations, plant water and nutrient relations with diminished crop productivity, and water use efficiency (WUE) (Li et al. 2009; Farooq et al. 2012).

Numerous drought indices have been developed in an attempt to identify, track, and describe drought events. Palmer Drought Severity Index (PDSI) uses precipitation, temperature, soil moisture and evapotranspiration factors on monthly calculation time scale (Alley, 1984). Mean temperature and precipitation are used in the weekly calculation of the Crop Moisture Index (CMI) (Palmer, 1968). Evapotranspiration is used to calculate the Crop Specific Drought Index (CSDI) on a seasonal time scale (Meyer et al., 1993). Standardized Precipitation Index (SPI) uses precipitation on 3-, 6-, 12-, 24- and 48-month periods calculation time scale (McKee et al., 1993). The

Standardized Precipitation Evapotranspiration Index (SPEI) uses precipitation and evapotranspiration on monthly calculation time scale (Vicente-Serrano et al., 2010). Standardized Runoff Index (SRI) uses precipitation factor on 3-, 6-, 12-, 24- and 48-month periods calculation time scale (Shukla & Wood, 2008) (Harsanyi et al., 2021).

2. Drought stress in sunflower

According to Salehi-Lisar and Bakhshayeshan-Agdam (2016), drought stress is one of the main obstacles to crop productivity in the twenty-first century and sunflower has no exception. Sunflower production is severely hampered by drought stress, which is a major obstacle to global sustainable crop production, particularly in arid and semi-arid countries (Wasaya et al., 2011). The common sunflower, *Helianthus annuus* L., is remarkable for its ability to survive in a variety of agronomic conditions, particularly in soils with varying water content (Raineri et al., 2015).

According to Hussain et al. (2013), sunflower has a strong root system that can absorb water from deeper soils. Additionally, the plant has a high capacity for photosynthetic growth since it has stomata on both sides of its leaves. However, because it grows mostly in tropical and subtropical climates, it is more vulnerable to drought, which reduces the seeds and oil yields (Hussain et al., 2018). Furthermore, water stress affects fatty acid content in addition to grain yields (Alberio et al., 2016, Howell et al., 2015).

The impact of drought on sunflowers is multi-leveled, with changes from phenotypic to physiological and biochemical indicators, including decreased plant height, leaf surface area, leaf relative water content, closed stomata and decreased levels of photosynthesis (Buriro et al., 2015), increased root length and the root-shoot ratio (Wu et al., 2022), shrinkage in cell volume, reduced water potential (Ghobadi et al., 2013), and membrane stability disrupted the balance of reactive oxygen (Soleimanzadeh, 2012).

Although, sunflower is a moderately drought tolerant crop, severe drought events causes reduction in the seed and oil yields. Therefore, it is crucial to understand the interaction between the physiological, biochemical, genetic, and agronomic bases of drought for its sustainable management to secure sustainable sunflower achene and oil production. Achene yield, oil quality, morphological and growth factors, as well as physiological and biochemical characteristics (such as photosynthesis, water relations, nutrient uptake, and oxidative damage) are all significantly impacted by drought stress in sunflower. Different management techniques, such as conventional or biotechnological breeding for drought tolerance, exogenous applications of hormones and osmoprotectants, seed treatments, and soil nutrient management have been suggested. Sunflower adjusts its osmotic balance, maintains its turgor, maintains its ability to absorb carbon, and controls its hormones in response to water stress. The enhancement of sunflower achene production and oil quality under drought stress requires thorough research on the integration of several management methods, including agronomic management, conventional breeding, and contemporary biotechnology advancements (Hussain et al., 2018).

Sunflower yield losses as a result of drought are significant (Prasad et al., 2008). According to Pekcan et al. (2015), exposure during the anthesis and dough stages in particular can cause crop losses of up to 80%. An important gene source for drought tolerance may be found in the wild relatives of domesticated sunflowers (Seiler et al., 2017). *Helianthus* is a sufficiently diverse genus, consisting of about 51 species and 19 subspecies (Vukich et al., 2009). In any water stress screening experiment, genotype characterisation based on relative water content, leaf water potential, photosynthetic efficiency, and proline concentration is essential (Darvishzadeh et al., 2011). Under drought stress, the amino acid proline is essential for both osmotic adjustments and free radical scavenging. Osmotic adjustments are important for the plants to undergo a smaller change in relative water content even with a decrease in water potential. This mostly helps plants to maintain cell expansion and growth even while it is under drought stress. Proline accumulation in drought-stressed sunflower is therefore viewed as an essential sign of the stressed genotypes' ability to resist drought (Cechin et al., 2006).

In contrast to seed filling, where restricted irrigation is tolerable, early- and mid-flowering phases are most affected by water deficit caused by limited irrigation, according to Karam et al. , (2007). According to Rauf, (2008), the 100-achene weight is reduced as a result of reduced photosynthesis driven by early leaf senescence during drought conditions.

3. Genetics and breeding for drought tolerance in sunflower

Finding the genes that truly affect drought tolerance among the vast number of genes is one of the most challenging tasks in sunflower genetic breeding (Wu et al., 2022). Drought tolerance is a complicated quantitative trait controlled by numerous genes. A sunflower hybrid experiences drought stress at three stages of growth (seedling, pre-flowering, and post-flowering) (Moschen et al., 2017). Using polyethylene glycol to replicate drought conditions for 24 hours, Liang et al. (2017) performed a transcriptome analysis on sunflower roots and leaves and discovered tissue specificity in gene expression, with more differentially expressed genes (DEGs) observed in leaves. Only one genotype of sunflower was employed in these trials (Wu et al., 2022). By comparing the phenotypes and transcriptome variations of various varieties grown under the same conditions, a better understanding of the drought-resistant response mechanism of sunflower can be gained, as demonstrated by the research of Sarazin Vivien et al., (2017).

The CMS PET-1 plant line is the most frequent source of cytoplasm genome in modern sunflower hybrids (Dimitrijevic & Horn, 2018). According to Tyagi et al. (2018), the homogeny in the cytoplasm of the majority of current hybrids may actually cause severe yield losses. This has already happened with other crops, such maize. The need for CMS source diversity is unavoidable in order to prevent such an undesirable consequence in sunflower. As a result, efforts have been made to diversify the cytoplasmic base, and a number of additional cytoplasmic sources have been discovered (Reddemann & Horn, 2018). Additionally, in certain cases, the detrimental consequences of the connection between the cytoplasmic and nuclear genomes led to a decrease in the amount of chlorophyll and the effectiveness of photosynthetic activity. In contrast, a positive effect of nucleus and cytoplasm interaction has also been reported for oil content. Therefore, the influence of cytoplasmic effects on important agronomic traits needs to be understood more precisely (Tyagi & Dhillon, 2017).

It is now possible to utilise genetic engineering to develop drought-tolerant sunflower via studies that have established methods for sunflower regeneration and genetic alteration (Radonic et al., 2015; Lewi et al., 2006; Manavella & Chan, 2009). Finding genes that confer drought tolerance on plants, however, is also necessary (Li et al., 2021).

The essential regulatory proteins known as transcription factors (TFs) have the power to activate or suppress whole transduction signal pathways. According to Giacomelli et al. (2010), WRKY family members of plant TFs have been identified as regulators of many developmental processes and linked to biotic and abiotic stress responses in sunflower. Plasticity is a trait of *Asteraceae* species (Panero and Funk 2008). They can be found across numerous biomes and are suited to a variety of soil types, harsh temperatures, and topographies (Timme et al. 2007). The sunflower HaWRKY6 gene, implicated in the temperature response and controlled by a miRNA, has been described as belonging to the WRKY family of plants (Giacomelli et al. 2012). Divergent transcription factors are particularly exciting proteins that need to be functionally characterised in order to understand how sunflower has adapted to such a wide range of environments. *Asteraceae* contains a novel branch of the WRKY family that is seemingly exclusive and diverse, and it contains the sunflower TF HaWRKY76, whose biological function is still unknown. This sister clade to the IId subgroup demonstrates at least three structural and possibly functional motifs that are absent from other clades' members (Giacomelli et al. 2010). To increase sunflower drought tolerance, scientists have recently focused more on the molecular biology that underlies the mechanisms of drought tolerance. For instance, Liu & Baird (2003) used differential display PCR and real-time quantitative PCR to examine the expression of five drought-induced genes in the roots, stems, and leaves of sunflower. Using a different strategy, Diaz-Martin et al. (2005) used gene interaction analysis to clone the drought-related DREB transcription factor HaDREB2 in sunflower. Using a cDNA array, Roche et al. (2007) examined the expression of genes involved in metabolism and signal transduction in sunflower leaves and immature embryos. The subsequent identification of 409 differentially expressed genes (DEGs), 82 of which were organ-specific and triggered by drought stress, revealed 409 DEGs. Additionally, Kane and Risesberg (2007) used selective screening to find candidate genes for sunflower drought and salt resistance and found 17 genes that were triggered by each of these conditions. Using an embryo rescue approach, Sauca and Lazar (2011) inserted the same drought tolerance gene into inbred lines of cultivated sunflowers. Furthermore, because the transcriptome of the sunflower is incomplete, the investigation of the molecular mechanisms behind drought resistance has generally concentrated on a small number of independent genes rather than a larger network of stress-induced genetic response genes. To understand the molecular mechanisms behind drought tolerance, one cannot focus on a single gene or gene class because drought tolerance is a complex phenotypic characteristic regulated by polygenes. Fortunately, transcriptome profiling and digital gene expression (DGE) analysis using high throughput sequence technology have facilitated discovery of DEGs under drought stress without relying on genome sequence information. Although annotation of DEGs is challenging without a genome sequence, the information acquired from DGE profiling could nonetheless describe the stress response mechanism to a certain extent (Liang et al., 2017).

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CHAPTER 6

RESPONSE CHANGES TO ENVIRONMENTS AND TRAIT PLASTICITY IN PLANTS

Dr. Ayşe Nuran ÇİL[1]

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[1] Republic of Türkiye Ministry of Agriculture and Forestry, Eastern Mediterranean Agricultural Research Institute, aysenuran.cil@tarimorman.gov.tr

1. Introduction

Functional traits are individual-level morphological, physiological, or phenological characteristics that have an indirect influence on plant performance and fitness through their impacts on growth, survival, and reproduction. Within species, trait expression is frequently flexible. The wide range of species performances in plant communities may be explained by functional features. Plasticity is found in all domains of life, but only recently there has been an increase in empirical studies.

The morphological, anatomical, physiological, biochemical, and phenological aspects of plants, referred to as plant traits, define how plants respond to environmental stimuli, influence other trophic levels, and alter ecosystem properties, as well as their advantages and disadvantages for humans. Therefore, information about plant traits serves as the basis for a broad field of research that includes evolutionary biology, community and functional ecology, biodiversity conservation, ecosystem and landscape management, restoration, biogeography, and earth system modelling (Kattge et al., 2020).

Living organisms have an amazing capacity to adapt their form and function in response to their surroundings. Phenotypic adjustments may occur on many different timescales, from durable and sometimes irreversible changes (developmental plasticity) to short-term, easily reversible responses variously labeled as contextual plasticity (Stamps & Groothuis, 2010), behavioural plasticity (Snell-Rood, 2013), or phenotypic flexibility (Piersma & Drent, 2003). Some individuals adapt to their environment by having significant phenotypic changes, whereas others are effected low. It is possible to study plasticity as a trait that emerges as a result of the genes of an individual acting in concert with their experiences in the past with their environment (Del Giudice, 2015).

An approach called the response-effect trait framework can be used to forecast how ecosystems will react to environmental change. To do this, the correct characteristics that explain a species' influence on ecosystem function (also known as "effect traits") and how that species reacts to environmental change (also known as "response traits") must be found. Response characteristics, like water availability, are frequently recognised and quantified along gradients in plant resources (Griffin-Nolan et al., 2018). Phenotypic plasticity, prevalent in all domains of life, enables organisms to cope with unpredictable or novel changes in their growing environment (Ashra & Nair, 2022). Species may be able to change their ecological strategy due to trait plasticity, allowing them to adapt to changing environmental conditions and avoid the ecological filter. It appears that both plants and animals exhibit stronger trait flexibility in invasive species than in native species (Funk, 2008; Berg & Ellers, 2010).

Genes, cells, tissues, as well as organisms and their environments all play a role in the development of phenotypes through causal interactions. It is frequently unclear how developmental and physiological systems will react to disturbances, such as when genes change or the environment changes, due to the complexity of these connections (Uller et al., 2018).

Theoretically, phenotypic plasticity can only evolve to a certain extent or may come at considerable costs because no creature is indefinitely or optimally plastic. For instance, plasticity may not be able to generate an optimal trait. Costs lead to reduced fitness when a trait is produced via plasticity rather than constitutively, and limits an inability to produce the optimal trait value (Murren et al., 2015).

By allowing organisms to produce phenotypes adjusted to the conditions that adults will experience, developmental plasticity can provide the means to cope with environmental heterogeneity. Developmental plasticity can be adaptive and its evolution can be shaped by natural selection (Lafuente & Beldade, 2019). When the environment changes, phenotypic plasticity causes a change in the phenotype, and this pre-existing plasticity is then refined by selection into an adaptable phenotype, this is known as plasticity-led evolution. The rate and degree of evolutionary change should be positively correlated with a phenotype's frequency of expression in a population, according to a key but largely untested prediction of evolution by natural selection. Essentially, the more often a phenotype is expressed and exposed to selection, the greater its opportunity for adaptive refinement (Levis & Pfennig, 2019).

However, the evolutionary significance of phenotypic plasticity is still controversial. According to a theory, flexibility prevents adaptation without genetic assimilation, which slows down evolution (Price et al., 2003). On the other hand, the so-called "flexible stem hypothesis" raises the possibility that a phase of plasticity may be a necessary step in the evolution of novel traits, wherein their expression at first remains conditional before it becomes fully integrated into the development and fixed (Gibert, 2017). It is unknown in how many instances the origination of novel traits has followed this pattern, because careful phylogenetic studies of novel traits using the comparative method are scarce. Nevertheless, by simultaneously using several developmental pathways, plasticity may accelerate evolutionary change. Since every alternative pathway is only expressed in a fraction of the population or in a limited number of generations, selective constraints are relaxed and mutations can accumulate more quickly, thereby accelerating evolution (Susoy et al., 2015). Together, this makes phenotypic plasticity an important concept in both developmental and evolutionary biology (Sieriebriennikov & Sommer, 2018).

2. Plant species

To establish a mechanistic knowledge of the variations in plant species performance under environmental change, trait-based approaches are essential. Although mean trait values have been used frequently to connect functional characteristics to species performance, it is still unknown what role intraspecific trait variation and trait plasticity play. Moreover, environmentally induced changes in species biomass can be caused by changes in the number of individuals and individual growth rate, both of which should be influenced by trait differences and plasticity (Zhang et al., 2020).

Functional attributes can be used to evaluate a species' plasticity (changes in behaviour, morphology, and physiology in response to the environment). According to Esperon-Rodriguez et al. (2020), these characteristics of plants have an impact on fitness through their effects on physiology, growth, reproduction, and survival. Climate has been demonstrated to have an impact on morpho-anatomical or physiological plant functioning characteristics. Physiological characteristics, or functional characteristics that operate to impart resistance or prevent acute or persistent water deficiencies, can be predictive of drought tolerance (Cornelissen et al., 2003). For instance, the leaf turgor loss point, also known as the wilting point, is regarded as a marker of drought resistance and is connected with water availability within as well as across biomes (Bartlett et al., 2014). Morpho-anatomical traits can also be correlated with water availability and with physiological traits (Esperón-Rodríguez et al., 2018). For instance, leaf morphotypes often change in response to prolonged water-limited conditions, resulting in smaller leaf size and reduced specific leaf area. Furthermore, drought tolerance has been shown to increase under hotter, drier conditions (Blackman et al., 2017).

Worldwide, biotic resistance serves as a significant natural defence against potential invaders, but the processes that underlie this resistance are still up for debate. Theoretically, native groups ought to defend themselves off both invaders with less competitive features and those that compete for the same resources but are functionally identical to them. Environmental stress, trade-offs between vital rates, and competition-induced plastic phenotypic alterations, nevertheless, may alter expected competitive outcomes, affecting invasion dynamics (Conti et al., 2018).

In order to link interspecific trait variation to species coexistence and performance under environmental change, species mean trait values, either measured in a given set of environmental conditions or obtained from a trait database (TRY, Figure 1) (Kattge et al., 2020), have been widely used (Griffin-Nolan et al., 2018). Recent research, for instance, suggests that species with resource-acquisitive traits—for example, shallow roots and thin leaves—are more responsive to environmental change because they can quickly acquire resources and outcompete species with resource-conservative traits when resources (for instance, water) are not scarce. In contrast, species with resource-acquisitive traits should suffer more than species with resource-conservative traits when resources become limiting (Conti et al., 2018).

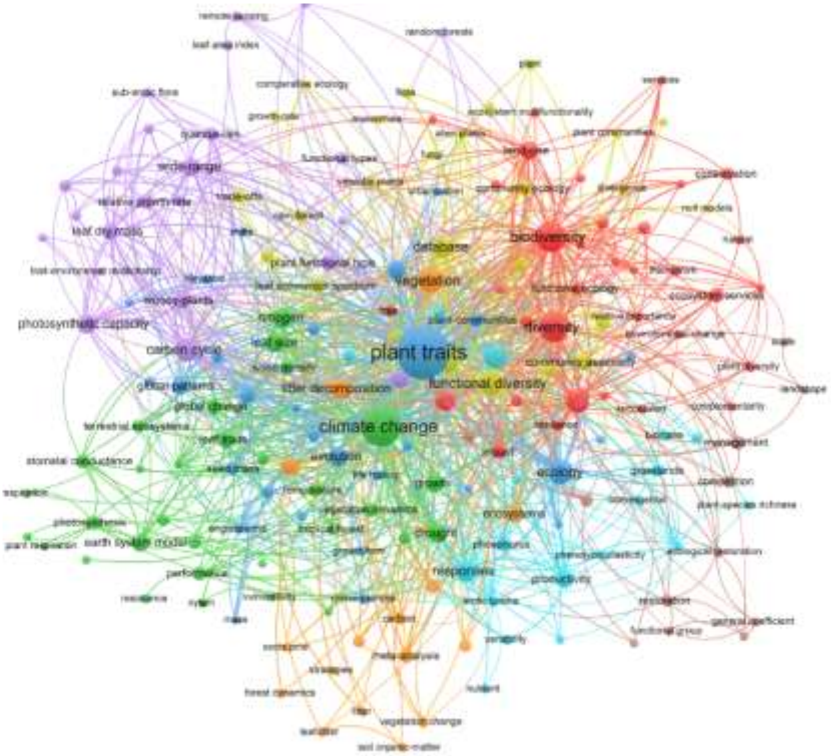

Fig. 1. Cluster analysis of keywords from peer-reviewed publications using plant trait data via TRY. Colours represent the eight clusters surrounding the central keywords (from largest to smallest cluster): biodiversity (red), climate change (dark green), plant traits (dark blue), functional diversity (light green), carbon cycle (violet), community (light blue), vegetation (orange), and environmental filtering (brown). The size of the circles and letters indicates the frequency of the keywords. 190 publications with DOIs retrieved by ISI Web of Science provide the basis for the analysis (Kattge et al., 2020).

A fascinating example of phenotypic plasticity that also directly reflects and influences the dynamics of biological interactions taking place in a community can be observed in plants. In response to the diverse host plant defences caused by herbivory, insects that interact with plants exhibit phenotypic plasticity in their developmental, physiological, morphological, or behavioural features. However, due to their dynamic relationship with their respective microbiomes and macrobiomes, plant-insect interactions are typically more complicated and multidimensional (Ashra & Nair, 2022).

Plants constantly modify their physiological, structural, and defensive characteristics in order to live and reproduce in settings with varying water, temperature, light, and harmful organism conditions because they are immobile (Des Marais et al., 2013). The variance in phenotypic plasticity between genotypes is thought to be responsible for the impact of genotype-environment interactions (GxE) on phenotypic variation (Huang et al., 2023). It has been demonstrated that phenotypic plasticity plays an essential role in the expression of phenotypic diversity in a variety of traits examined in different environments (Kusmec et al., 2018).

3. Plant genetics and breeding

Plants are extraordinary because they can survive despite having a limited ability to modify their environment. In particular, plants are exceptional at detecting, responding to, and surviving a wide range of abiotic challenges. The changes in physiology, metabolism, growth, and uncertain development that occur as a result of acclimatisation to the environment are mostly responsible for this. These reactions are frequently measured and represented as reaction norms, and they can be thought of as the capacity of a single genotype to develop various phenotypes in response to the environment (Des Marais et al., 2013).

In eukaryotes, polyploidy, or whole-genome duplication frequently accompanied by hybridization, is widespread and is regarded to be a major factor in ecological and evolutionary success, particularly in plants (Wei et al., 2019). The repeated and widespread occurrence of polyploidy throughout the plant kingdom reflects its widespread adaptive significance (Van de Peer et al., 2017). While polyploidy is common in eukaryotic lineages, some of the best-known examples of polyploids in flowering plants include important crops (Salman-Minkov et al., 2016) and many invasive species (te Beest et al., 2012). Due to chromosomal duplication and the flexibility of gene expression, polyploidy may change the functional trait plasticity (Jackson & Chen, 2010; Madlung & Wendel, 2013). Relative to diploids, polyploids can potentially employ alternative copies of duplicated genes gained from diverse and possibly adaptive genetic backgrounds to respond to novel environments (Dong & Adams, 2011; Shimizu-Inatsugi et al., 2017). Thus, it is hypothesized that polyploids can exhibit higher trait plasticity than diploids in response to varying environment (Wei et al., 2019).

The epistasis model, the pleiotropy model, and the overdominance model are the three genetic models for phenotypic plasticity that have been proposed (Huang et al., 2023). According to the epistasis concept, the interaction between the genes that control the mean phenotype and linear plasticity results in the plastic response to environmental inputs. According to the pleiotropy concept, phenotypic plasticity is brought on by the same genes' differential expression, which affects the mean phenotype in different environments. According to the overdominance model, the plastic response is caused by the degree of heterozygosity of a given gene. If the pleiotropy model is supported, breeders may need to make trade-offs between mean phenotype values and plasticity, whereas if the epistasis model is supported, breeders can increase mean phenotype values while also increase suitable plasticity (Kusmec et al., 2017).

There is considerable interest in the occurrence and molecular mechanisms of phenotypic plasticity and genotype-by-environment interactions (G×E) in plant populations. The emergence of genomic tools, including quantitative trait locus (QTL) mapping and transcriptome studies, provides opportunities to identify G×E patterns and mechanisms across a diversity of phenotypes, species, and environments. G×E is common at both the QTL and gene expression levels and is primarily driven by differential sensitivity of alleles. Numerous natural variations and mechanisms have been connected to this phenomenon at the molecular level. These include, but are not limited to, nonsynonymous changes in receptor proteins (PhyB, Cry2), loss-of-function mutations in transcriptional repressors (FRI, FLC), splicing variations in biosynthetic enzymes (P5CS1), and gene duplication in transcription factors (Sub1A) (Des Marais et al., 2013).

Multidimensional analyses of data from genomic screens of transcript, protein, or metabolite profiles may thus provide some of the first insights into the integrated structure of plastic responses and a better understanding of constraints (Walsh & Blows, 2009). One approach in crop breeding is to reduce plasticity in order to develop a cultivar that performs satisfactorily in a variety of conditions (Lynch and Walsh, 1998). As an alternative, it is possible to enhance performance in some situations by amplifying advantageous alleles that are conditionally neutral or unfavourable in other environments (Xiao et al., 2022). This is similar to how natural selection has acted on wild populations, in which local adaptation has resulted in genotypes with optimized phenotypes in their native environments that are often maladapted in new environments (Blanquart et al., 2013; Jin et al., 2023).

Individual environmental stresses that parents experince can have an impact on their offspring's phenotypes and improve their survival in similar environments. This transgenerational plasticity were first thought to directly reflect resource levels; resource-poor parents producing low-quality offspring as a result of decreased provisioning (e.g., to seeds or eggs). In some cases, stressed parents produce offspring with specific developmental alterations that mitigate that particular type of stress (Herman & Sultan, 2016). These changes lead to heritable, environmentally driven adaptability when these offspring experience similar circumstances (Walsh et al., 2016). Although such adaptive transgenerational plasticity has been thoroughly demonstrated, it is usually unknown how it is transmitted. DNA methylation alterations caused by the environment and heritable factors represent one possible mechanism (Kappeler & Meaney, 2010). The presence or absence of methyl groups on cytosine residues can affect the transcriptional activity at particular loci in several taxa. Numerous environmental factors, including biotic stresses like pathogen infection (Pastor et al., 2013) and abiotic stresses like drought (Alsdurf et al., 2016), can cause alterations to DNA methylation throughout the genome in plants. Such genotype-dependent environmental methylation alterations have been demonstrated to be heritable in a number of species, often for numerous generations (Dubin et al., 2015; Feil & Fraga, 2012). Furthermore, heritable methylation variation can have substantial impacts on ecologically important traits. For instance, investigations of epigenetic recombinant inbred lines in *Arabidopsis thaliana* demonstrated that, in the absence of DNA sequence diversity, DNA methylation variants can significantly contribute to heritable variation in important parameters like primary root length and flowering time. DNA methylation is a leading prospective mechanism for adaptive transgenerational plasticity, especially in plants, due to its genotype-specific environmental sensitivity, transgenerational stability, and phenotypic impact (Herman & Sultan, 2011). Yet a central question remains unresolved: to what extent do genotypes in natural populations vary in their epigenetic responses to stressful parental environments (Schaefer & Nadeau, 2015; Herman & Sultan, 2016)?

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CHAPTER 7

MAGNESIUM DEFICIENCY IN PLANTS

Dr. Neriman Tuba BARLAS[1]

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[1] Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ege University, 35100 İzmir, Türkiye tuba.barlas@ege.edu.tr, tubabarlas@gmail.com, Orcid ID: 0000-0002-2971-4977

Introduction

Magnesium (Mg) is an essential plant nutrient, and its deficiency may be observed worldwide. It has various significant functions, such as participating in the structure of the chlorophyll molecule and taking part in photosynthesis, carbohydrate, lipid, and energy metabolisms.

The fact that it plays a role in such a wide area in plant metabolism may cause inhibitions and decreases in both yield and quality in case of deficiency. Failure to sustain needed amount of Mg in time often cause irreversible damages, especially on fruit quality. In order to better understand the deficiency symptoms, knowing the functions of the relevant nutrient in plant metabolism supports the diagnosis and often provides early solutions to be sought.

Magnesium concentrations in plants

Magnesium generally varies between % 0.15 and 1.00% in plants. Although plants whose leaves contain less than 0.2% Mg are mostly considered to be Mg-deficient; this limit can often take quite different values depending on the specie/variety, part and development stage of the plant (Embleton, 1966; Mengel and Kirkby, 1978; Bould et al., 1984; Scaife and Turner, 1984; Bose et al., 2011; Hauer-Jákli and Tränkner, 2019). Adequate Mg concentrations for some plant groups are presented in Table 1.

Table 1. Critical Mg concentrations for some plants[1]

Plant groups	Sufficiency level of Mg (%)	
	Based on dry matter formation	Based on optimum photosynthetic activity
Woody plants	0.09–0.40	0.10–0.50
Monocots	0.07–0.21	0.15–0.41
Dicots	0.10–0.70	0.10–0.72

[1]Modified from (Römheld, 2012; Hauer-Jákli and Tränkner, 2019).

Critical Mg concentrations are generally lower for monocotyledonous than dicotyledonous plants (Wilkinson et al., 1990). Generally, leaves from plants containing more than 0.4% Mg (dry weight) are deemed adequate in Mg.

According to other research sufficiency level of Mg concentration in leaf (dry weight) varies from 0.1 to 0.2% in rice, wheat, maize, barley, sorghum, and potato. There is a higher requirement of Mg for the dry matter formation in soybean, cotton or peanut within the range of 0.2-0.3%, and even much higher 0.35% in the tomato, sunflower and alfalfa (Römheld, 2012; Hauer-Jákli and Tränkner, 2019). Sufficiency levels of Mg for some plant species are presented in Table 2.

Table 2. Sufficiency levels of Mg in plants based on their tissue sampled[1]

Species	Tissue sampled	Deficient (%)	Adequate (%)
Citrus spp.	Leaf	<0.15	0.30-0.70
Beta vulgaris L.	Petiole	0.01-0.03	0.10-0.70
Beta vulgaris L.	Blade	0.025-0.05	0.10-2.50
Zea mays L.	Blade	0.07	>0.20
Brassica oleracea L.	Top	0.43	0.56-0.57
Cucumis sativus L.	New leaf	<0.35	0.50-0.90
Lactuca sativa L.	Head	0.05-0.10	0.24-0.48
Lycopersicon esculentum Mill	Leaf	0.12-0.19	0.40-0.60
Cynodon dactylon L.	Tops	0.10	0.15-0.60
Festuca arundinacea Schreb.	Tops	0.08	0.15-0.60
Medicago sativa	Tops	0.30	0.30-0.70
Stylosanthes	Tops	0.27	>0.30
Acer	Leaf	0.03-0.09	>0.09

[1]Modified from Embleton (1966), Mengel and Kirkby (1978), Bould et al. (1984), Scaife and Turner (1984), Wilkinson and Mays (1979), Wilkinson et al. (1987), Winsor and Adams (1987), Plucknett and Sprague (1989)

Common Causes of Mg deficiency in Plants

The availability of Mg to plants depends on various factors such as low Mg contents in source rocks (Papenfuß and Schlichting, 1979); pH- texture- cation exchangeable capacity and clay type of soils, (Hariadi and Shabala, 2004; Marenco and Lopes 2011), cationic competitions, site specific climatic and anthropogenic factors, growth practices, as well as plant's intrinsic characters related to its specie and variety (Scheffer and Schachtschabel, 2002; Mikkelsen, 2010; Wang et al., 2020).

Deficiency of magnesium occurs particularly in plants growing in highly leached acid soils with low cation exchange capacity (Schubert, et al., 1990). The underlying reason for the frequent occurrence of Mg deficiency in such soils is that leaching carries the Mg from the root environment to the lower layers, making the root zone poorer in terms of magnesium (Tan et al., 1991; vanPraag et al., 1997; Aitken et al., 1999; Bose et al., 2011). Moreover, a competition between H^+ and Al^{3+} in the absorption of Mg^{2+} can occur in acid soil. The reason for the competition between Mg^{+2} and H^+ and Al^{+3} in acidic soils is explained by the relatively weak binding of Mg^{+2} to negatively charged groups in the stem cell walls. Also, another competition between Mg^{2+} and Ca^+ can also be found in soils having higher pH via limestone applications in high doses. Thus, by lowering or raising the pH, the absorption of Mg^{2+} decreases due to competition from H^+ or Al^{3+} or Ca^{2+} (Fernandes et al., 2018, Ferreira et al., 2023).

It is also reported that magnesium is in exchangeable form in the soils having a pH lower than 6.0 while it turns into a non- exchangeable form at the soil pH reaching a value higher than 6.5 (Chan et al., 1979; Hailes et al., 1997).

Clay type, soil texture and parent material are also significant factors affecting Mg availability/deficiency to plants. For example, peridotite and dolomite basaltic rocks and chlorite, vermiculite and illite clays are rich in magnesium (Marenco and Lopes, 2011).

Plants growing in light soils with low organic matter and in soils derived from Mg-poor rocks may suffer from Mg deficiency. Soils having magnesium less than 6% or having Mg^{2+} less than 48 mg dm^3 are assumed to be Mg-insufficient (Ferreira et al., 2023). Intensive crop production is also reported as one of the reasons for Mg depletion in soils (Pol and Traore, 1993).

The cationic competition may occur as a result of low or high values of soil pH as above mentioned. However, long-term imbalanced soil fertilization may also be a factor on cationic competition. Excess K in fertilization can induce Mg deficiency especially in plants in need of higher amounts of potassium. Magnesium deficiency is also induced when the Ca/Mg ratio becomes too high, as the plant can absorb less magnesium (Ferreira et al., 2023).

Symptomatology

Visual diagnosis is an effective tool to assess the symptoms of a nutritional disorder (deficiency or toxicity) by the appearance of the plant. In most cases, the color change of the leaves is the most obvious signal (Prado et al., 2010). Magnesium deficiency symptoms first occur in older leaves as it is mobile in the phloem. In severe cases, symptoms may be seen in younger leaves as well.

It is well known that its symptoms may vary depending on the species and/or cultivar. The most characteristic sign of its deficiency is an interveinal chlorosis which can be described as a yellowish color while the leaf veins remain green. Chlorosis may be delayed in the leaves as mesophyll cells close to the vascular bundles keep chlorophyll stable for a longer period than parenchyma cells. Moreover, tan or reddish colors may also occur depending on the specie/cultivar.

A lower amount of the yield after the leaf falls is also observed (Novais et al., 2007). A delay in the reproductive phase in addition to accumulation of non-structural carbohydrates such as starch and sugars, less translocation of carbohydrates from the aerial part to the root and impairment of the root developments which may result in a lowered root absorption of plant nutrients are counted as the other common signs of the Mg deficiency (Cakmak et al., 1994; Taiz and Zeiger, 2004; Nèjia et al., 2016; Adnan et al., 2021).

Effects of Magnesium Fertilization on Some Crops

Azarmi et al., (2015) investigated the effects of different Mg concentrations on cucumber (*Cucumis sativus* L.) in solution form in hydroponic culture under low (50%) and optimum (100%) light intensity. As a result of the research, fruit firmness increased with the application of 1 mM Mg and with the increasing light intensity. However, growth and fruit quality are enhanced with Mg (3 mM) applied at optimum light intensity.

Hao & Papadopoulos, (2003) conducted an experiment on the effects of increasing doses of Ca (150 mg and 300 mg Ca/L) and Mg (20, 50, 80 and 110mg Mg/L) treatments on tomatoes (*Lycopersicon esculentum* Mill.) grown in rockwool. At the bottom and mid of the leaves after eight weeks of planting showed the leaf chlorosis at the 20mg Mg/L. Under the low concentrations of Mg (20 mg Mg/L) 50% of the leaves having moderate chlorosis lost their photosynthetic capacity and showed a delay in fruiting. Under low Ca, firmness of fruit enhanced with increasing Mg doses.

Zlamalova et al., (2015) studied the individual (3,86 kg Mg ha^{-1} and 12.44 K ha^{-1}) and combined (1.93 kg Mg ha^{-1} with 6.22 kg K ha^{-1}) effects of foliar application of magnesium sulphate and potassium sulphate during a three-year experiment (2011-2013). As a result of their study, it is seen that individual application of Mg and K have significant effects on increasing the yield.

El-Zohiri and Asfour, (2009) investigated the effects of individual and combined foliar applications of Mg, K ad Ca on yield and some yield components of potato (*Solanum tuberosum* L.). As a result of their study, it is concluded that foliar applied potassium sulphate combined with calcium nitrate or with magnesium has significant effects on number and weight of tubers and yield amount as well.

Al-Barzinji and Naif, (2014) conducted an experiment to evaluate the effects of different forms ($MgSO_4.7H_2O$, $Mg(NO_3)_2.6H_2O$, $MgCl_2.6H_2O$) of foliar Mg applications in garlic (A*llium sativum* L.) At the end of the study, highest number of the leaves was found by $MgCl_2$, highest amount of dry matter of shoots was found by $Mg(NO_3)_2$. The foliar application of $MgSO_4$ and $Mg(NO_3)_2$ enhanced the head diameter and weight, yield amount and quality of garlic.

A research was conducted to understand the effects of foliar Mg (0.25, 0.50 and 0.75% Mg) applications on cauliflower (*Brassica oleracea* var. botrytis). At the end of the experiment, it is seen that Mg improves the fresh weight and height of the plant, fresh and dry weight of the leaves and total production of curds (Ahmed et al., 2011).

Herman et al., (2004) conducted an experiment to evaluate the effects of Mg on sugarbeet. As a result of their experiment, an accumulation of sucrose in the fully expanded leaves were found in Mg-deficient plants.

In a comprehensive research the effect of Mg fertilization on various species was evaluated. At the end of the research, it was concluded that Mg application significantly enhanced the yields in many species with an average value of 8.5% (Wang et al., 2020). Detailed information is given in Table 3.

Table 3. The rates of yield increase after Mg applications in various species (%)[1]

Unit	Species									Average
	Others[2] (46)[3]	Tea (12)	Oil Crops (46)	Cereals (165)	Vegetables (49)	Tubers (67)	Tobacco (76)	Grasses (28)	Fruits (52)	
%	1.5	6.9	8.2	8.2	8.9	9.4	9.8	10.6	12.5	8.5

[1]Modified from Wang et al. (2020), [2]This group were not affected significantly by the Mg applications, [3] Numbers indicated in parentheses refer the sample size.

Conclusion

Magnesium is one of the key factors in agricultural production where its deficiency may result in severe problems such as reductions in yield and product quality. This is why, to take the whole plantation under control is quite critical to avoid any possible Mg deficiency or to fix the deficiency problem(s) via foliar and/or soil fertilization on time. Even if any hunger sign of Mg is seen; a reliable fertilization with a correct timing may help to reduce and to regress the deficiency symptoms in plants.

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CHAPTER 8

PHENOLIC METABOLISM IN PLANTS UNDER HEAVY METAL STRESS

PhD Student Vesile YALCIN[1]
Assoc. Prof. Dr. Hülya TORUN[2]

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[1] Duzce University, Graduate School of Education, Department of Biology, Duzce, Türkiye. yalcinvesile42@gmail.com, ORCID ID: 0000-0003-1293-732X

[2] Duzce University, Faculty of Agriculture, Department of Biosystem Engineering, Duzce, Türkiye. hulyatorun@duzce.edu.tr, ORCID ID: 0000-0002-1118-5130

1. INTRODUCTION

In the present era, rapidly increasing environmental pollution poses a great threat to various ecosystems (Adrees et al., 2015). Heavy metals (HMs), which are among the environmental problems, spread to the environment as a result of geogenic sources and anthropogenic activities and constitute one of the main components of toxic pollutants in the world (Liu et al., 2018; Mansoor et al., 2021). HMs refer to both essential micronutrients (e.g., Cu, Fe, Zn) and non-essential metals/semi-metals (metalloids) (e.g.,Cd, Pb, As, Cr). (Malik et al., 2022). Their endurance and non-biodegradation of their in nature cause bioaccumulation and biomagnification in water and soil (Anjitha et al., 2021; Fu et al., 2021). This poses a threat to the natural biogeochemical cycle and food chain (Haider et al., 2021; Hussain et al., 2021).

It has been reported that there is soil pollution in more than 10 million areas worldwide, and more than 50% of this pollution is contaminated with heavy metals and metalloids (He et al., 2015). Toxic metal ions, which are usually taken up by plants in chelated and complex forms, adversely affect the growth, development and metabolic processes of plant (Boechat et al., 2016; Malav et al., 2020). Toxic levels of metals/metalloids cause various phytotoxic damages such as abnormalities in biomolecules such as DNA, RNA, lipids, proteins, increased reactive oxygen species (ROS), decreased photosynthetic activity and stomatal conductance, membrane peroxidation, enzyme inhibition (Shahid et al., 2014; Emamverdian et al., 2015; Bukhari et al., 2016; Singh et al., 2019; Mondal et al., 2022). In response to HMs stress, plant cells use defense mechanisms such as hormones, phytochelatins, metal tolerance proteins, metallothioneins, reactive nitrogen species, chelating metal ions, detoxifying the vacuole, antioxidant defense system and secondary metabolites (Rahim et al., 2022; Niyoifasha et al., 2023).

Phenolic compounds (PCs) constitute the largest group of plant-specialized metabolites with more than eight thousand components (González-Sarrías et al., 2020; Samec et al., 2021). PCs are found in roots, seeds, flowers and edible parts of plants (de la Rosa et al., 2019; Rochetti at al., 2022). Phenolics are important signalling molecules produced under optimal and sub-optimal conditions in plants and play a role in hormonal regulation, cell division, photosynthetic activity, nutrient mineralization and reproduction (Sharma et al., 2019). PCs are naturally produced from the aromatic amino acid phenylalanine via the shikimic acid-malonic acid biosynthetic pathway (Lee et al., 2017; Zhang et al., 2022). PCs are characterized by one or more hydroxyl groups directly attached to at least one benzene ring (Lund, 2021). The hydroxyl group in the structure of phenols acts as a metal chelator that binds to metal ions, renders them harmless, and reduces the translocation of metal ions to the above-ground part (Kısa et al., 2016; Ghori et al., 2019). At the same time, PCs are important antioxidants involved in scavenging ROS, oxidative damage to DNA, proteins, and lipids (Gawlik-Diziki et al., 2012). Increased PC biosynthesis in plant tissues protects plants against toxic metal stress and helps them adapt to unfavorable environments (Kisa et al., 2016; Ahmad vd., 2018; Safari vd., 2019; Chen et al., 2020; Lwalaba et al., 2020; Antony ve Nagella, 2021; Janczak-Pieniazek vd., 2023).

2. CLASSIFICATION AND STRUCTURE OF PHENOLIC COMPOUNDS

Phenolic metabolism is one of the highly branched networks that provide about 40% of the organic compounds in plants (Kováčik and Klejdus, 2008). Phenolics are second only to hemicellulose, cellulose and lignin and are the fourth major component in plants (Yan et al., 2021; Chen et al., 2023). These compounds are often found in the cell walls and vacuoles of epidermal and subepidermal cells in plants (Kisiriko et al., 2021). They are organic compounds synthesized during the normal development of plants and in response to stressful conditions (Shahidi et al., 2019). The family of PCs is secondary metabolites showing different chemical structures and reactivity, ranging from simple compounds to highly polymerized compounds (Gómez-Caravaca et al., 2014). Compounds containing at least one hydroxyl group attached to the benzene ring are called simple phenols, compounds containing more than one hydroxyl group are called polyphenols (Kougan et al., 2013; Silva et al., 2023). PCs or polyphenols are classified according to the number of benzene rings in their structure and the structural elements that connect these rings to other rings (Zengin, 2015). They are divided into four main groups according to their chemical structure: phenolic acids, flavonoids, lignans and stilbenes (Spencer et al., 2008).

2.1. Phenolic Acids

The main feature of all phenolic acids is that they are attached to a benzene ring or a carboxyl group (Samec et al., 2021). Phenolic acids usually exists in the form of glycosides, esters, or amides, rarely in free form **(**Hu et al., 2021). Phenolic acids are divided into two classes such as hydroxybenzoic and hydroxycinnamic acid according to their structures (Sun and Shahrajabian, 2023). Hydroxybenzoic acids are found attached to cell structural components, glycosyl moieties and small organic molecules with C6-C1 skeletons (Kisiriko et al., 2021). Gallic, salicylic, syringic, protocatechuic, *p*-hydroxybenzoic, and vanillic acid are common hydroxybenzoic acids (de la Rosa et al., 2019; Kisiriko et al., 2021). Hydroxycinnamic acids have a C6-C3 skeleton and often bind to molecules such as glucose and quinic acid. Caffeic, ferulic, sinapic, and p-coumaric acids are the main hydroxycinnamic acids (Kumar and Goel, 2019; Bodoira and Maestri, 2020). Hydroxycinnamic acid derivatives are more common than hydroxybenzoic acid derivatives and are found in almost all plants (El-Seedi et al., 2012).

2.2. Flavonoids

It was first isolated from orange in 1930 (Silva et al., 2023). Nowadays, they are the most abundant polyphenols in plant foods, with more than 4000 compounds (Birt and Jeffery, 2013). The basic structure of flavonoids consists of a C6-C3-C6 skeleton. These phytochemicals are generally stored in vacuoles in the form of glycosides (Shen et al., 2022). Plant flavonoids are natural bioactive compounds abundant in vegetables, fruits, cereals and tea (Shen et al., 2022). These compounds give colour to fruits and flowers and play an important role in pollination, defence and reproductive functions (Cahyana and Adiyanti, 2021). Flavonoids have a wide range of compounds such as flavanols, flavonols, flavanones, flavones, isoflavones, anthocyanins due to variations in the hydroxylation pattern and oxidation state of the central pyran ring (Singla et al., 2019). Flavones have a double bond between C2-C3 and a ketone at the C4 position and apigenin, luteolin are the most important flavones (Panche et al., 2016). Flavonols, unlike flavones, contain a hydroxyl group at the C3 position and quercetin, kaempferol are the most well-known (Singla et al., 2019). Flavanols are characterised by a hydroxyl group at the C3 or C4 position without a double bond between C2-C3 and catechin, epicatechin is the most abundant (Luo et al., 2022). Flavanones, the double bond between the C2-C3 positions are saturated and naringenin, hesperidin are the most common (Panche et al., 2016). Isoflavonoids are precursors in the development of phytoalexins, mostly found in the Fabaceae family, and genistein, daidzein are the best known (Panche et al., 2016; Bernatoniene et al., 2021). Anthocyanins are distinguished from other flavonoids by the presence of two double bonds in their heterocyclic rings (Singla et al., 2019). Anthocyanins are natural plant pigments found in fruit and flower tissues that exhibit a wide range of colours from pink-orange to red, purple and blue hues (Santos-Beulga and González-Paramás, 2019). The most widely studied anthocyanins are cyanidin, delphinidin, pelargonidin, peonidin and malvidin (Panche et al., 2016). Their sugar-bound units are glucose, arabinose and galactose, e. g. (Singla et al., 2019).

2.3. Lignans

Lignans are a large group of polyphenols derived from the oxidative coupling of two C6-C3 units (Teponno et al., 2016). Most of these compounds are found in free form in nature, while a few are present as glycosides (Cui et al., 2020). They are indispensable substances for secondary cell wall thickening and there are about 3000 different lignan structures (Schmidt et al., 2010). Lariciresinol, matairesinol, medioresinol, secoisolariciresinol, syringaresinol, pinoresinol are the main lignans found in plants (Durazzo et al., 2018). They can be found in many different parts of the plant such as roots, stems, leaves, flowers, fruits, seeds, xylem, resin and rhizomes (Cui et al., 2020). Lignans are found in various plant materials such as vegetable oils, seeds (sesame, linseed), legumes (broccoli, soya beans), especially in plants rich in fibre (Silva et al., 2023).

2.4. Stilbenes

Stilbenes are characterized by two phenyl moieties linked together by a two-carbon chain in a C6C2C6 skeleton structure (Peng et al., 2018; Silva et al., 2023). Stilbenes exist in both *cis* and *trans* isomeric forms as well as free (minor) and glycosylated (major) forms (Singla et al., 2019). Nowadays more than 400 stilbene derivative compounds have been identified (Khawand et al., 2018). Resveratrol is the most studied stilbene due to its high bioavailability

(Valletta et al., 2021). Stilbenes are natural products synthesized by plants in response to external influences such as pathogenic attacks, wounding and UV radiation (Teka et al., 2022). They act as phytoalexin (response to infection) in some plant families (Langcake and Pryce, 1977).

3. PHENOLICS in COUNTERACTING HEAVY METAL PHYTOTOXICITY

Phenolic compounds are important phytochemical defence mechanisms involved in the coping of plants with various HMs stress conditions. The present book chapter provides an overview of phenolic compound responses to the effect of some toxic metal stress (As, Cr, Cd, Pb, Ni, Cu) on the physiological and metabolic processes of plants.

3.1. Cadmium (Cd) Phytotoxicity

In the study in which different doses (3, 6, 9, and 12 mg $CdCl_2$ kg^{-1}) of Cd were applied to *Zea mays* plants, it was found that total anthocyanin content increased at all Cd doses (Hussain et al., 2013). In another study, Cd toxicity increased the content of rutin, cinnamic acid derivatives and epicatechin in *Erica andevalensis* (Márquez-García et al., 2012). Cd (120 µM) exposure to *Matricaria chamomilla* plant caused accumulation of cinnamate derivatives (Kováčik et al., 2009c). Similarly, it was determined that exogenous Cd application increased the total phenolic and flavonoid content *of Withania somnifera* (Mishra and Sangwan, 2019). In another study, increased flavonoid content in *Avicennia marina* roots was found to increase tolerance to Cd (Li et al., 2015). Cd application to Zea mays plants caused a significant increase in the content of chlorogenic acid and rutin, whereas it caused a decrease in the content of ferulic acid and caffeic acid (Kisa et al., 2016). In another study in which the relationship between Cd stress and phenolic metabolism of *Kandelia obovata* plant was determined, it was determined that increasing Cd concentration increased the total phenolic compound content in leaves and roots (Chen et al., 2019). Phenolic compounds showed a protective effect against Cd toxicity in *Vaccinium corymbosum* plant grown in a medium with different concentrations of Cd (Manquián-Cerda et al., 2016). *Malva parviflora* plants treated with Cd (40 µM) showed an increase in total flavonoids and total phenolic content (Zoufan et al., 2020). In bread wheat grown under cadmium-induced toxicity (25 and 50 µM $CdCl_2$), the protective role of anthocyanins was observed in the coleoptiles of seedlings at 25 µM Cd dose, while anthocyanins were ineffective against stress at 50 µM Cd dose (Shoeva and Khlestkina, 2018). In a recent study, it was determined that Cd application at different concentrations (10, 20, and 50 ppm) caused a significant decrease in caffeic, chlorogenic, vanillic acid and total phenolic levels in tomato plants (Kısa et al., 2019). In the other study, phenolic compound content increased in *Zea mays* plants grown in the Cd-contaminated sand medium (Hassan et. al., 2022).

3.2. Chromium (Cr) Phytotoxicity

Increased the contents of total phenols, flavonoids and anthocyanins in *Brassica juncea* plants grown in Cr-contaminated soil (300 μMKg^{-1}) helped to protect the plant from oxidative stress by reducing Cr toxicity (Handa et al., 2019). In another study, an increase in stilbene and anthraquinone metabolites was observed in the roots of *Fallopia × bohemica* plants exposed to Cr (VI) toxicity (Barberis et al., 2020). Under Cr (VI) toxic metal stress, phenolic and flavonoid content of rice is higher in roots than in shoots (Dubey et al., 2018). Phenolic and flavonoid contents of tolerant and sensitive *Zea mays* plants grown in Cr-contaminated sand were higher in Cr-tolerant cultivars (CZP, Pearl, Sahiwal) than in Cr-sensitive cultivars (MMRI, EV-77, Sadaf) (Hassan et. al., 2022). The application of quercetin to *Trigonella corniculata* plants grown in Cr-toxic soil was reported to alleviate Cr toxicity (Aslam et al., 2022). Cr at different concentrations (25, 50, 75, and 100 ppm Cr per kg of soil) in *Portulaca oleracea* increase in the amount of nonenzymatic antioxidant compounds (phenolic content and flavonoids) (Rahbarian et al., 2019). Endogenous and root-exuded phenolic compound content decreased significantly in sunflower seedlings grown under Cr-induced stress conditions (Qadir et al., 2020). In another study, Canola (*Brassica napus* L.) was grown under Cr toxicity (15 and 30mg/kg soil) and shoot phenolic content increased at both Cr concentrations (Khan et al., 2021).

3.3. Lead (Pb) Phytotoxicity

The content of phenolic acids and flavonoids such as ferulic acid, vitexin, daidzein and salicylic acid increased in *Prosopis farcta* plants exposed to Pb stress (Zafari et al., 2016). A positive correlation was observed between total phenolic and chlorogenic acid content in *Zea mays* plants exposed to Pb (Kisa et al., 2016). Metal stress increased flavonoid content in 'Hyacinth' cultivar of winter wheat treated with Pb (Janczak-Pieniazek et al., 2023). In a study, five different doses of Pb (0, 2500, 5000, 7500, and 10000 ppm) were applied to *Atriplex canescens* and an increase in total polyphenol and flavonoid content was observed in leaves and roots (Ikram et al., 2020). In another study, Pb-treated *Lupinus luteus* showed tolerance to toxic metal stress by increasing flavonoid content (Izbianska et al., 2014). Exogenous naringenin treatment was reported to alleviate Pb stress in mung bean (Sharma et al., 2021). In the present study, *Polygonum aviculare* (L.) and *Senecio vulgaris* (L.) plants, a positive correlation was observed between Pb exposure and polyphenol and flavonoid activity in plant shoots and metal concentration. (Salinitro et al., 2020). Similar results, total phenol and total flavonoid levels at increasing Pb concentration were observed in *Mentha aquatica* L., *Eryngium caucasicum* (Trautv.) and *Froriepia subpinnata* (Ledeb.) plants (Hasanpour et al., 2022).

3.4. Copper (Cu) Phytotoxicity

Increased biosynthesis of phenols and flavonoids showed protective effect of *Panax ginseng* against Cu stress (Ali et al., 2006). Another study, it was observed that Cu application to *Lycopersicon esculentum* plants increased phenolic compounds and flavonoid levels (Chakraborty et al., 2015). Cu (1000 ppm) treatment of *Triticum aestivum* plants increased the flavonoid content in "Hyvento" cultivar (Janczak-Pieniazek et al., 2023). Cu exposure increased the content of phenolic compounds (vanillic, caffeic and gallic acid) and flavonoids (kaempferol-3-O-glycosides and rutin) in *Prosopis glandulosa* leaves, causing the plant to tolerate metal stress (Gonzalez-Mendoza et al., 2018). In another study, it was observed that *Prosopis glandulosa* leaf epidermal polyphenol content was not affected by Cu stress (Gonzalez-Mendoza et al., 2017). The total phenolic and flavonoid content of *Adhadota vasica* and *Withania somnifera* plants in Cu-contaminated soil exhibited a maximum for *A. vasica* and a minimum for *W. somnifera* (Maharia et al., 2012). In a recent study, flavonoids play an important role in the survival of soybean plants in Cu-contaminated soil (Chernyshuk et al., 2020). Similar result was revealed with increased phenolic and flavonoid content in castor seedlings that withstand the Cu stress (Sameena and Puthur, 2022).

3.5. Arsenic (As) Phytotoxicity

Application of As (300 µmol L^{-1}) showed an increase in both total phenolic and anthocyanin content in basil. (Dehabadi et al., 2013). In another study, As treatment increased the content of acetic acid, citric acid, fumaric acid, malic acid, formic acid and oxalic acid in *Spinacia oleraceae* L. parallel to the increasing As concentration (Saleem et al., 2022). Anthocyanin accumulation in C3 (barley) and total phenolic and flavonoid accumulation in C4 (maize) plants treated with nano-sized arsenic oxide nanoparticles (As_2O_3-NP) were observed (Selim et al., 2021). Similar result was recorded in *Vicia faba* plants treated with As (100, 200 and 400 µM) with an increase in total phenolic fraction (Mohamed et al., 2016). Another experiment, *Raphanus sativus* L. plants treated with three different As concentrations (0.3, 0.5, 0.7 mM) decreased content of flavonoid, anthocyanin and phenolic (Bhardwaj et al., 2023). In a study investigating selenium (Se)-mediated As tolerance in rice seedlings, a significant increase in the levels of phenolic compounds such as ferulic acid, gallic acid, protocatechuic acid was determined (Chauhan et al., 2017).

3.6. Nickel (Ni) Phytotoxicity

Selenium (Se) application to *Lactuca sativa* plants exposed to Ni stress increased anthocyanin content (Hawrylak et al., 2007). *Matricaria chamomilla* was exposed to Ni for 10 days, total soluble phenolic content did not change in leaf rosettes, while total phenolic content increased in roots (Kováčik et al., 2009a). *Fagopyrum esculentum* was treated with Ni at different time intervals (24h, 48h, 72h). According to the results of this study, an increase in caffeic, *p*-anisic, hesperetic, chlorogenic, chlorogenic, *p*-hydroxybenzoic levels in leaves after 24h and 48h Ni application, and a decrease in hesperetic, *p*-anisic, *p*-hydroxybenzoic, affeic, p-coumaric acids content after 72h Ni exposure were observed (Sytar et al., 2013). In another study, it was determined that the increase in the amount of non-enzymatic antioxidants such as phenolic compounds and flavonoids in *Eclipta prostrata*, which was applied Ni, protected against the harmful effects of the toxic metal (Chandrasekhar and

Ray, 2019). In another recent study, Ni exposure induced the phenolic content of *Pisum sativum* (El-Amier et al., 2019).

4. CONCLUSION AND FUTURE PROSPECTIVE

Toxic trace metals adversely affect crop yield and quality worldwide due to their detrimental effect on plant growth and development. These metals pose a risk for many physiological and biochemical reactions such as ROS production, electrolyte leakage, lipid peroxidation, photosynthesis rate. Increased biosynthesis of phenolic phytochemicals has an important role in scavenging free radicals, maintaining membrane stability, osmoregulation, induction of antioxidant enzyme activity and metal chelation. On the other hand, translocation of toxic metals taken up by the roots to the above-ground parts may pose a danger to human and animal health through the food chain as a result of the popularity of herbal medicines, herbal raw materials, the safety of medicines, dry and fresh consumption of plants. Therefore, the discovery of stress-resistant species is important for cleaning agricultural areas from pollutants, preventing food safety problems in the future, producing crops without compromising crop quality and for a sustainable environment. Also, the exogenous use of phenolic phytochemicals is also important in terms of being a natural antioxidant source alternative to synthetic antioxidants. Therefore, much more work is needed to confirm the protective role of phenolic metabolism in plant growth under different environmental conditions and toxic metal species.

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CHAPTER 9

INOCULANTS, ENZYMES, AND OTHER ADDITIVES IN SILAGE PRODUCTION: A REVIEW OF THE LITERATURE

Assist. Prof. Dr. İbrahim ERTEKİN[1]
Prof. Dr. İbrahim ATIŞ[2]

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[1] Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, Hatay
E-mail: ibrahimertekin@mku.edu.tr Orcid. 0000-0003-1393-8084
[2] Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, Hatay
E-mail: iatis@mku.edu.tr Orcid. 0000-0002-0510-9625

1. INTRODUCTION

Silage is a type of fermented animal feed made from green plants that have been harvested and stored in a manner that allows them to ferment (Ertekin and Kızılşimşek, 2020). The fermentation process is carried out by microorganisms, mainly lactic acid bacteria and yeasts, which convert the plant sugars into lactic acid (Kung et al., 2018). This acidification process preserves the feed and reduces the risk of spoilage, making silage an important source of feed for livestock (Muck, 2010).

The quality of silage depends on a number of factors, including the initial quality of the plant material, the fermentation process, and the storage conditions (Villa et al., 2020). The quality of the plant material is influenced by factors such as the species and stage of growth of the plants, the presence of contaminants, and the moisture content (Zhang et al., 2020). The fermentation process is influenced by factors such as the pH, temperature, and presence of specific microorganisms (Czatzkowska et al., 2020). The storage conditions, including the type of storage container and the duration of storage, can also affect the quality of silage (Daniel et al., 2019).

Poor-quality silage can lead to reduced feed intake and reduced animal performance, while high-quality silage can improve animal health and productivity (Niderkorn and Jayanegara, 2021). In addition to its role in animal nutrition, silage has important environmental and economic impacts, as it can reduce the need for synthetic fertilizers and reduce the amount of food waste (Holm-Nielsen et al., 2009).

Silage fermentation is a complex process that is influenced by a number of factors, including the pH, temperature, moisture content, and presence of specific microorganisms (Yan et al., 2019). The pH of silage is an important factor that affects the rate and efficiency of fermentation, as well as the final quality of the product (Da Silva et al., 2017) Silage pH is typically in the range of 3.5-4.5, with a lower pH being associated with more rapid and efficient fermentation (Dou et al., 2022). The pH of silage can be influenced by the pH of the plant material, the presence of specific microorganisms, and the amount of lactic acid produced during fermentation (Fabiszewska et al., 2019).

The presence of specific microorganisms can also affect the rate and efficiency of silage fermentation. Lactic acid bacteria, such as *Lactobacillus* and *Pediococcus*, are the main microorganisms responsible for the fermentation of silage (Ávila and Carvalho, 2020). These bacteria are able to tolerate high concentrations of lactic acid and can survive in the low pH environment of silage (Ávila and Carvalho, 2020). Inoculants, which are commercial preparations containing specific strains of lactic acid bacteria, are often used to improve the fermentation of silage (Weinberg et al., 2004). Enzymes, such as cellulases and hemicellulases can also be added to silage to improve the fermentation process by breaking down plant cell walls and releasing sugars (Taye and Etefa, 2020).

The impact of different additives on silage quality can be complex and may depend on the specific additive and the conditions of the fermentation process. Inoculants have been shown to improve the fermentation of silage and can increase the concentration of lactic acid and decrease the pH, resulting in improved silage quality (Wang et al., 2022; Li et al., 2022). Enzymes may also improve the fermentation of silage by increasing the release of sugars from plant cell walls, which can be fermented by lactic acid bacteria (Ding et al., 2022). However, the impact of enzymes on silage quality may be affected by other factors, such as the type and amount of enzyme used, the stage of plant growth, and the storage conditions (Yang et al., 2022a). Some studies have found that the addition of enzymes to silage can improve the nutritional value of the product, while others have found no significant effect (Yang et al., 2022a; Ren et al., 2021; Irawan et al., 2021; Elwakeel et al., 2007).

In conclusion, the rate and efficiency of silage fermentation are influenced by a number of factors, including the pH, temperature, moisture content, and presence of specific microorganisms. The addition of additives, such as inoculants and enzymes, can improve the fermentation of silage and may have a positive impact on silage quality. However, the specific impact of these additives on silage quality may be affected by a range of factors, and the use of some additives is regulated due to potential health and environmental concerns.

1.1. Overview of the silage fermentation process and the microorganisms involved

The silage fermentation process begins with the ensiling of the plant material, which involves cutting and wilting the plants to reduce their moisture content, and then storing them in a sealed container (Ertekin et al., 2022). The plants are usually stored in a silo or a plastic bag, and the absence of oxygen in the container allows the lactic acid bacteria to grow and ferment the plant sugars (Carvalho et al., 2021).

Lactic acid bacteria are the main microorganisms responsible for the fermentation of silage (Zhao et al., 2021). These bacteria are facultative anaerobes, meaning that they can grow in the presence or absence of oxygen. They are able to produce lactic acid from various sugars, including glucose, fructose, and sucrose (Coelho et al., 2022). The most common lactic acid bacteria found in silage are species of the genera *Lactobacillus* and *Pediococcus*. These bacteria are able to tolerate high concentrations of lactic acid and can survive in the low pH environment of silage (Kuppusamy et al., 2020).

In addition to lactic acid bacteria, yeasts and fungi may also be present in silage. Yeasts, such as species of the genera *Saccharomyces* and *Pichia*, can contribute to the fermentation process by converting sugars into ethanol (Nandal et al., 2020). Fungi, such as *Aspergillus* and *Penicillium*, can produce mycotoxins, which are toxic compounds that can pose a risk to animal health (Kępińska-Pacelik and Biel, 2021). The presence of fungi in silage is generally considered to be a sign of poor-quality silage (Kung et al., 2018).

The pH of silage is an important factor that affects the rate and efficiency of fermentation, as well as the final quality of the product. Silage pH is typically in the range of 3.5-4.5, with a lower pH being associated with more rapid and efficient fermentation (Dou et al., 2022). The pH of silage can be influenced by the pH of the plant material, the presence of specific microorganisms, and the amount of lactic acid produced during fermentation (Soundharrajan et al., 2021).

Temperature is another important factor that influences silage fermentation. Optimal fermentation occurs at a temperature of about 15-20°C, with higher or lower temperatures potentially leading to slower or less efficient fermentation (Du et al., 2022). Moisture content is also an important factor, as silage with a moisture content above 60% may be more prone to spoilage due to the growth of aerobic microorganisms (de Almeida Carvalho-Estrada et al., 2020). In contrast, silage with a moisture content below 40% may be difficult to ferment due to the low water activity.

The presence of specific microorganisms can also affect the rate and efficiency of silage fermentation. Lactic acid bacteria, such as *Lactobacillus* and *Pediococcus*, are the main microorganisms responsible for the fermentation of silage (Guan et al., 2020). These bacteria are able to tolerate high concentrations of lactic acid and can survive in the low pH environment of silage (Li et al., 2021). Inoculants, which are commercial preparations containing specific strains of lactic acid bacteria, are often used to improve the fermentation of silage. Enzymes, such as cellulases and hemicellulases, can also be added to silage to improve the fermentation process by breaking down plant cell walls and releasing sugars (Chaji et al., 2020).

The final product should have a pH of about 3.5-4.5 and a dry matter content of about 20-40%. The presence of yeasts and fungi in silage may be an indication of poor-quality silage, as these microorganisms may produce compounds that are toxic to animals or that affect the nutritional value of the feed (Kępińska-Pacelik and Biel, 2021; Kung et al., 2018). The nutritional value of silage is influenced by factors such as the protein content, digestibility, and energy content of the feed.

Silage fermentation is an important process that preserves the feed and reduces the risk of spoilage, making it an important source of feed for livestock. The quality of silage depends on a number of factors, including the initial quality of the plant material, the fermentation process, and the storage conditions (Kung et al., 2018). Poor-quality silage can lead to reduced feed intake and reduced animal performance, while high-quality silage can improve animal health and productivity (Niderkorn and Jayanegara, 2021). In addition to its role in animal nutrition, silage has important environmental and economic impacts, as it can reduce the need for synthetic fertilizers and reduce the amount of food waste (Xu et al., 2021).

1.1.1. Inoculants and enzymes

Inoculants are commercial preparations containing specific strains of lactic acid bacteria that are used to improve the fermentation of silage. These products are typically applied to the plant material at the time of ensiling (Kung et al., 2018) or pre-harvesting (Ertekin and Kızılşimşek, 2020), and the bacteria in the inoculant help to establish the lactic acid fermentation process (Muck, 2010). Inoculants can be composed of single or multiple strains of lactic acid bacteria, and they may also contain enzymes to improve the fermentation process (Filya, 2013).

The use of inoculants in silage production has been shown to improve the fermentation process and increase the concentration of lactic acid, leading to improved silage quality. Inoculants can also reduce the pH of silage, which can inhibit the growth of spoilage microorganisms and reduce the risk of silage spoilage (Kung et al., 2018). In addition, inoculants may improve the nutritional value of silage by increasing the concentration of lactic acid, which can improve the digestibility of the feed (Bernardi et al., 2019).

Enzymes are proteins that catalyze chemical reactions in the body (Winkler et al., 2018), and they can also be added to silage to improve the fermentation process (Xing et al., 2009). Enzymes such as cellulases and hemicellulases can break down plant cell walls and release sugars that can be fermented by lactic acid bacteria (Chaji et al., 2020). The use of enzymes in silage production has been shown to improve the fermentation process and increase the concentration of lactic acid, leading to improved silage quality (Guo et al., 2014). However, the impact of enzymes on silage quality may be affected by other factors, such as the type and amount of enzyme used, the stage of plant growth, and the storage conditions (Muck, 1988). Some studies have found that the addition of enzymes to silage can improve the nutritional value of the product, while others have found no significant effect (Yang et al., 2022b; Colombatto et al., 2004; Bolsen et al., 1996; Chen et al., 1994).

In conclusion, inoculants, enzymes, and other additives can have a positive impact on the fermentation of silage and the quality of the final product. Inoculants can improve the fermentation process and increase the concentration of lactic acid, leading to improved silage quality. Enzymes can break down plant cell walls and release sugars that can be fermented by lactic acid bacteria, although the impact of enzymes on silage quality may be affected by other factors. Preservatives and antimicrobials can also improve the quality of silage by inhibiting the growth of spoilage microorganisms, although the use of these additives is regulated due to potential health and environmental concerns.

1.2. A general evaluation according to literature review

Overall, the literature suggests that the pH, temperature, moisture content, and presence of specific microorganisms can all affect the rate and efficiency of silage fermentation (Li et al., 2022; Ávila and Carvalho, 2020; Kung et al., 2018). Lower pH and optimal temperature (around 15-20°C) are generally associated with more rapid and efficient fermentation (Du et al., 2022), while high moisture content and the presence of aerobic microorganisms can lead to slower or less efficient fermentation (Ávila and Carvalho, 2020).

The use of inoculants, which are commercial preparations containing specific strains of lactic acid bacteria, has been shown to improve the fermentation of silage and increase the concentration of lactic acid, leading to improved silage quality (Carvalho et al., 2021). The use of enzymes, such as cellulases and hemicellulases, may also improve the fermentation process by breaking down plant cell walls and releasing sugars that can be fermented by lactic acid bacteria (Li et al., 2019). However, the impact of enzymes on silage quality may be affected by other factors, such as the type and amount of enzyme used, the stage of plant growth, and the storage conditions (Yang et al., 2022a). Some studies have found that the addition of enzymes to silage can improve the nutritional value of the product (Oliveira et al., 2019; Khota et al., 2018; Wang et al., 2019; Queiroz et al., 2012) while others have found no significant effect (Ren et al., 2021; Weideet al., 2020; Bureenok et al., 2019).

Other additives, such as preservatives and antimicrobials, may also be used to improve the quality of silage (Zhang et al., 2022a; Wu et al., 2020). Preservatives, such as propionic acid and formic acid, can inhibit the growth of spoilage microorganisms and reduce the risk of silage spoilage (Song et al., 2022; Jiang et al., 2020; Zhang et al., 2019). Antimicrobials, such as antibiotics and organic acids, can also inhibit the growth of spoilage microorganisms and may improve the fermentation of silage (Kaewpila et al., 2020). However, the use of these additives is regulated and may be limited in some countries due to concerns about the potential impact on animal health and the environment (Pandey et al., 2019).

In summary, the rate and efficiency of silage fermentation are influenced by a number of factors, including the pH, temperature, moisture content, and presence of specific microorganisms (Yan et al., 2019). The addition of additives, such as inoculants and enzymes, can improve the fermentation of silage and may have a positive impact on silage quality. However, the specific impact of these additives on silage quality may be affected by a range of factors (Kung et al., 2018), and the use of some additives is regulated due to potential health and environmental concerns.

The findings from the literature review on the factors that influence the rate and efficiency of silage fermentation and the impact of different additives on silage quality have important implications for the silage industry and for the production of high-quality silage. For the silage industry, understanding the factors that affect silage fermentation and the use of additives to improve silage quality can help to optimize the production process and improve the efficiency of silage production. In addition, the use of high-quality silage can improve animal health and productivity, which can have important economic benefits for farmers and other stakeholders in the industry.

For the production of high-quality silage, it is important to consider the pH, temperature, moisture content, and presence of specific microorganisms, as these factors can influence the rate and efficiency of fermentation and the final quality of the product. In addition, the use of inoculants and enzymes may improve the fermentation process and the quality of the final product. However, the specific impact of these additives on silage quality may be affected by a range of factors, and the use of some additives is regulated due to potential health and environmental concerns.

In addition to the factors that influence the rate and efficiency of silage fermentation, it is also important to consider the nutritional value of silage. The nutritional value of silage is influenced by factors such as the protein content, digestibility, and energy content of the feed (Oba and Kammes-Main, 2022; Khalilian et al., 2022; Adhianto et al., 2020; Ertekin and Kızılşimşek, 2020; Kızılşimşek et al., 2016), and it can have a significant impact on animal health and productivity (Valdez-Arjona and Ramírez-Mella, 2019; Abd El-Hack et al., 2018). High-quality silage can provide a valuable source of nutrients for livestock, while poor-quality silage can lead to reduced feed intake and reduced animal performance.

The use of additives, such as inoculants and enzymes, may also affect the nutritional value of silage. Some studies have found that the addition of these additives can improve the nutritional value of silage (Zhang et al., 2022b; Carvalho et al., 2021; Su et al., 2019), while others have found no significant effect (Tian et al., 2022; Gao et al., 2021; Vu et al., 2019). It is important to carefully consider the impact of additives on the nutritional value of silage and to use additives in a responsible manner to ensure that the final product is of high quality and meets the nutritional needs of livestock.

In addition to its role in animal nutrition, silage has important environmental and economic impacts. Silage production can reduce the need for synthetic fertilizers and reduce the amount of food waste, as it allows farmers to preserve excess plant material and use it as feed. Silage can also help to reduce the reliance on purchased feed, which can have economic benefits for farmers.

In conclusion, the findings from the literature review on silage microbiology have important implications for the silage industry and for the production of high-quality silage. Understanding the factors that affect silage fermentation and the nutritional value of silage, as well as the impact of additives on these factors, can help to optimize the production process and improve the efficiency of silage production, leading to improved animal health and productivity. In addition, silage has important environmental and economic impacts, and its production can help to reduce the need for synthetic fertilizers and reduce the amount of food waste. The findings from the literature review on silage microbiology have important implications for the silage industry and for the production of high-quality silage. Understanding the factors that affect silage fermentation and the use of additives to improve silage quality can help to optimize the production process and improve the efficiency of silage production, leading to improved animal health and productivity.

2. CONCLUSION

Main points of the review on silage microbiology:

- Silage is a type of fermented animal feed made from green plants that have been harvested and stored in a manner that allows them to ferment.
- The fermentation process is carried out by microorganisms, mainly lactic acid bacteria and yeasts, which convert the plant sugars into lactic acid.

- The pH, temperature, moisture content, and presence of specific microorganisms can all affect the rate and efficiency of silage fermentation.
- The use of inoculants, enzymes, and other additives may improve the fermentation process and the quality of the final product.
- The nutritional value of silage is influenced by factors such as the protein content, digestibility, and energy content of the feed.
- Silage has important environmental and economic impacts, and its production can help to reduce the need for synthetic fertilizers and reduce the amount of food waste.

Some areas for future research in the field of silage microbiology:

- Further investigation of the role of different microorganisms in silage fermentation: While lactic acid bacteria and yeasts are known to play a major role in silage fermentation, there is still much to be learned about the specific strains of these microorganisms that are most effective in silage production, as well as the roles of other microorganisms such as acetic acid bacteria and heterofermentative bacteria.
- Development of new additives to improve silage quality: There is ongoing research into the development of new additives, such as enzymes and probiotics, that can improve the fermentation process and the quality of the final product. Future research could focus on identifying new additives that are more effective or have fewer potential negative impacts on animal health and the environment.
- Investigation of the impact of silage additives on the nutritional value of the final product: While some studies have found that additives such as inoculants and enzymes can improve the nutritional value of silage, further research is needed to fully understand the mechanisms behind these effects and to identify the optimal use of these additives in terms of dosage and timing.
- Exploration of the role of silage in sustainable livestock production: Silage production can have important environmental and economic benefits, including the reduction of food waste and the reliance on purchased feed. Future research could focus on the potential for silage

to contribute to more sustainable livestock production systems, including the use of alternative feedstocks and the potential for silage to reduce greenhouse gas emissions.

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CHAPTER 10

SOME CHEMICALS USED IN CUT FLOWER VASE SOLUTIONS AND THEIR EFFECTS

Dr. Veysel AYDIN[1]

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[1] Batman University, Sason Vocational School, Department of Plant and Animal Production, Batman veysel.aydinzm@batman.edu.tr 0000-0001-9496-7711

1. INTRODUCTION

The ornamental plants sector in the world has been developing rapidly in recent years. Depending on this growth, the cut flower sector is also growing day by day. However, the ornamental plants sector has many problems from the production stage to the marketing stage. The short post-harvest strength of cut flowers is an important problem in the marketing sector.

Cheap labor and climate advantages have caused cut flower production areas to spread to many parts of the world. This situation has extended the delivery time of cut flower products from the producer to the final consumer. The fact that production and consumption centers are far from each other has made it more important both to preserve the vitality of flowers and to prevent product losses.

The vase life of cut flowers is defined as the time from when the stems are placed in the vase solution until they lose their visual quality (Halevy and Mayak, 1981).

The main factors affecting the post-harvest life of cut flowers are; genetic structure, growing conditions, growing season, environmental conditions, harvest time, harvesting method, the processes applied during and after the harvest and the processes applied while the flower is in the vase (Orçun and Erdem, 1973).

Dilley and Carpenter (1975) reported that the durability of cut flowers after harvest can be extended by using some chemicals. Various solutions are used to increase the post-harvest strength of cut flowers. Water, sugar, germicides, plant growth regulators, ethylene inhibitors and pH regulators are commonly used in these solutions. These substances can be used alone or together, and some substances may have more effects. It has been reported that high doses of the compounds used in vase solutions can cause toxic effects (Van Doorn et al., 1990). For this reason, it is important to know the amounts and effects of the substances used.

2. INGREDIENTS IN VASE SOLUTIONS

2.1 Water

Cut flowers begin to lose water from the moment they are harvested. As a result of the deterioration of the water balance of the flowers which cannot receive enough water, bent-neck, wilting and yellowing occur. Cut flowers get the water they lose from the vase water they are placed in. The clean water needed for the continuation of the metabolic activity is important in terms of preventing bacterial formation and blocking of the vascular bundles.

Blocking of the vascular bundles and deterioration of the water balance in cut flowers cause a shortening of the vase life (Uzun et al., 1983). It has been reported that germicides used in vase solutions are effective in prolonging the vase life by increasing the water uptake of flowers (Jones and Hill, 1993).

Treating cut roses with hot water after harvest helps to escape air from the vascular bundles and to prevent oxidation in the vascular bundles (Kuhlen, 1958). Durkin and Kuc (1966) reported that low ambient humidity increases transpiration in flowers, thus negatively affecting water uptake and reducing vase life.

2.2 Sugar

Sugars are widely used in solutions used to extend the vase life of cut flowers. The sugars used in vase solutions regulate the movement of stomata, thus reducing water loss through transpiration. Sugars provide the continuation of metabolic activity and delay the events that develop due to aging (Morousky et al., 1968; Goszczynska et al., 1988).

Sugars are effective by regulating the plants water balance and osmosis (Uzun et al., 1983). There have been reported in many studies that % 2-10 sucrose is effective in prolonging the vase life of cut flowers (Nowak, 1981; Ketsa and Narkbua, 2001; Çelikkol, 2008; Aydın, 2015). In addition to these positive effects of sucrose, there are also some negative effects of it. It has been reported that due to the increase in the amount of sugar added to the vase water, the water intake decreases and the bacterial density in the vase water increases (Aydın, 2015).

2.3 Germicides

The most important factor in the rapid wilting of cut flowers is the blocking of the vascular bundles due to physiological reasons and the activity of microorganisms. The main microorganisms that block the vascular bundles of cut flowers are *Bacillus spp., Pseudomonas spp., Fusarium spp., Enterobacter spp*. (Kuhlen et al., 1958; Put et al., 1988). It has been reported that bacterial growth in vase water causes blocking in the transmission bundles of cut roses and affects the reduction of water intake (Aydın, 2015).

Microorganism growth in vase solutions in many cut flower species significantly reduces the vase life of flowers. When germicides (fungicide and bactericide) known to have a reducing effect on microbial growth are added to the vase solution, the vase life is prolonged (Larsen et al., 1967; Morousky, 1968; Burdett, 1970; Lineberger and Steponkus, 1976; Morousky, 1977; Mayak et al., 1977; Van Meeteren, 1978).

Germicides and fungicides are added to vase solutions at appropriate doses for different flower types in order to prevent blocking in the transmission bundles. These include silver nitrate, silver thiosulfate, 8-hydroxyquinoline citrate, 8-hydroxyquinoline sulfate, thiobendazole, aluminum sulfate, quaternary ammonium substances, slowly decomposing chlorinated substances (Nowak and Rudnicki, 1990; Damunupola and Joyce, 2008). Some germicides and application doses used in vase solutions are shown in table 2.1.

Table 2.1 Some germicides used in vase solutions and their application doses (Mengüç, 1991).

Compound Name	Application Dose
8-hydroxyquinoline sulfate (8-HQS)	200-600 ppm
8-hydroxyquinoline citrat (8-HQC)	200-600 ppm
Silver nitrate (SN)	10-200 ppm
Silver thiosulfate (STS)	0.2-4 mM
Thiobendazole (TDZ)	5-300 ppm
Quaternary ammonium substances (QAS)	5-300 ppm
Chlorinated substances (CS)	50-400 ppm
Aluminum sulfate (AS)	200-300 ppm

8-Hydroxyquinoline is a flower preservative chemical compound widely used in cut roses at doses up to 200-600 mg/L (Nowak and Rudnicki, 1990; Van Doorn, 1997).

Kazaz et al. (2019) reported that the application of 300 ml/L 8-hydroxyquinoline prolongs the vase life of cut hydrangea flowers.

Nowak (1981) investigated the effects of pretreatment on the vase life of cut gerbera flowers. In the study, it was reported that 8-hydroxyquinoline sulfate (200 mg/L) solution prolongs the vase life, reduces breakage and bent-neck in the stems of flowers.

Hashemabadi et al. (2015) investigated the effects of pennyroyal (*Mentha pulegium*) extract and 8-hydroxyquinoline sulfate applications on cut roses. In the study, they used pennyroyal extract at doses of %0, 10, 20 and 30, and 8-hydroxyquinoline sulfate at doses of 0, 200, 400 and 600 mg/L. In the study, they obtained the longest vase life from the application of 8-HQS (400 mg/L) + pennyroyal extract (10%).

Ichimura et al. (2002) investigated the effects of different preservatives on the vase life of cut roses. The longest vase life was determined by the solution containing 200 mg/L 8-HQS+20 g/L sucrose.

Elgimabi and Ahmed (2009) investigated the effects of 8-HQS and sucrose on the vase life of the cut rose. In the study, 8-HQS was used at doses of 100, 200 and 300 ppm. The longest vase life was observed in 100 ppm 8-HQS application.

When silver ions are added in the vase solution, they are carried to the tip of the flower. Flowers with silver added to vase water live longer than flowers without added silver (Kader et al., 1985).

It has been reported that nano silver particles obtained by using nano technology have an antimicrobial effect at low concentrations, antibacterial properties are related to the surface area of nanoparticles, and that the decrease in particle size and therefore the increase in surface area provides more effect for antibacterial activity (Baker et al., 2005). It has been reported that nano silver application is effective in prolonging the vase life of cut roses and preventing bacteria occurrence in vase water (Aydın, 2015).

Lü et al. (2010) investigated the effects of nano silver particles on the vase life of the cut rose. In the study, cut rose stems were pulsed in nano silver at doses of 50, 100 and 250 mg/L. The longest vase life was obtained from 50 and 100 mg/L nano silver pulsing applications. They determined that the number of bacteria at the bottom of the stems increased in all applications during the vase life, and a lower number of bacteria was detected in the nano silver application compared to the control.

Butt (2005) examined the effects of different solutions on the vase life of the cut roses. The researcher placed some of the cut roses in pure water after pulsing in 50, 100 and 150 ppm $AgNO_3$ for 24 hours. In the study, the longest vase was measured in 150 ppm $AgNO_3$ solution.

Aluminum sulfate, silver nitrate, silver thiosulfate and sodium thiosulfate are bactericides commonly used in cut flower preservative solutions. Silver thiosulfate reduces the negative effect of ethylene (Nowak and Rudnicki, 1990; Arboleda, 1993).

Gherghi et al. (1983) examined the performance of some rose cultivars in preservatives. The longest vase life was determined in the solution containing 4% sucrose + 0.1% aluminum and potassium sulfate + 0.02% potassium chloride.

2.4 Plant Growth Regulators

Plant growth regulators are used before and after the harvest of cut flowers. It has a positive effect on the prevention of leaf yellowing and water uptake in cut flowers.

Cytokinins increase the water uptake of flowers and accordingly increase the turgor pressure in the petals. In this way, they are effective in maintaining post-harvest quality (Mor et al., 1983).

Goszczynska et al., (1985) determined that cytokinin is beneficial in maintaining the postharvest quality of cut flowers such as rose, carnation, gerbera, chrysanthemum, tulip, anthurium and iris.

Leaf yellowing causes a decrease in post-harvest quality in cut flowers. Leaf yellowing emerges as an important problem in some cut flower species such as solidago, lily, chrysanthemum, alstromeria and gillyflower (Kazaz, 2015; Çelikel, 2020). Leaf yellowing causes the decreasing of the quality of the solidago flowers and the shortening of the vase life (Aydin et al., 2022).

Leaf yellowing can be controlled by the use of plant growth regulators at various concentrations (Thomas and Stoddart, 1980; Thimann, 1985). The effect of the products used against leaf yellowing varies according to the species and cultivars. Gibberellins (GA_3) and cytokinins (benzylaminopurine, zeatin) are sprayed or used as solutions (Philosoph-Hadas, 1996; Hassan et al., 2003; Çelikel, 2013).

The effects of benzyl adenine and gibberellic acid treatments against leaf yellowing in cut lily flowers were investigated. In study, it was determined that the leaf yellowing in lilies could be significantly delayed by the application of gibberellic acid (250 mg/L) and benzyl adenine (50 mg/L). It has been reported that benzyl adenine and gibberellic acid applications before cold storage are more effective than after storage (Han, 1995).

Funnell and Heins (1998) examined the effect of plant growth regulators on leaf yellowing of potted asiflorum lilies. In study, 0, 50, 250 and 500 mg/L Promalin (gibberellic acid A_4/A_7 + benzyl adenine 1:1) and Accel (gibberellic acid A_4/A_7 + benzyl adenine 1:10) were applied to the plants by spraying. The 250 mg/L dose of Promalin application completely eliminated the leaf yellowing during 20 days.

Ferrante et al. (2005) examined the effects of benzyladenin (BA) and thidiazuron (TDZ) on leaf yellowing. In the study, chrysanthemum varieties sensitive to leaf yellowing (Regan bianco) and non-sensitive (Regan giallo) were used. BA 50 and 100 mg/L, TDZ 5 and 10 mg/L doses were used in the study. Cut chrysanthemum flowers were pulsed for 24 hours in the prepared solutions. According to the results, both cytokines delayed leaf yellowing in Regan bianco cultivar. It was also found that the effect of TDZ was stronger and significantly increased the total leaf chlorophyll content by 35% compared to the initial value.

Philosoph-Hadas et al. (1996) investigated the effects of silver thiosulfate (GTS), naphthalene acetic acid (NAA), gibberellic acid (GA_3) and benzyladenine (BA) in order to prevent leaf yellowing and improve quality of solidago flowers. According to the results obtained from the research, it was determined that GTS and BA were effective in prolonging the vase life and preventing leaf yellowing. However, they reported that GTS and BA were more effective when used together.

2.5 Ethylene Inhibitors

Ethylene plays an effective role in growth and development process synthesized in plants. Controlling ethylene in cut flower species sensitive to ethylene is important in terms of preserving their post-harvest quality.

Ethylene shortens vase life by causing flower, leaf and petal shedding, yellowing of leaves, necrosis and aging. Apart from aging of petals and leaves, it also affects bud opening. Therefore, anti-ethylene products are widely used in ethylene-sensitive cut flowers.

In vase solutions, silver thiosulfate, 1-methylcyclopropane aminooxyacetic acid, aminoethoxyvinylglycine and potassium permanganate are used as anti-ethylene.

It has been reported that silver ions not only reduce bacterial activities, but also have ethylene inhibitory properties (Halevy and Kofranek, 1977; Halevy and Mayak, 1981; Nichols and Sussex, 1982; Uzun et al., 1983; Mengüç and Türk, 1984).

By inhibiting ethylene synthesis, the post-harvest life of cut flowers can be extended. Therefore, silver thiosulfate is used as an ethylene inhibitor in postharvest solutions (Kazaz et al., 2020).

Silver ions are substances that have both antibacterial and anti-ethylene effects. It prevents the development of microorganisms in vase solutions and flower stems. Silver also prevents ethylene synthesis and delays aging (Goszczynska et al., 1988).

Demircioğlu (2010) examined the effects of 1-MCP application on the vase life of the “First Red” cut rose variety. In the study, it was determined that different 1-MCP (control, 100 nl/l and 200 nl/l) applications were effective on the vase life of First Red roses. It has been reported that 1-MCP applied roses extend the storage period and are effective in maintaining visual quality.

2.6 pH Regulators

The vase life of cut flowers is related to the use of chemicals that inhibit the growth of bacteria and fungi in vase solutions. In this respect, copper and silver compounds reduce the pH of the vase water and prevent the growth of bacteria and fungi (Orçun and Erdem, 1973).

It is desirable that the pH value of the water or solution is low (3.5-5.0) in the post-harvest water extraction of cut flowers (Dole and Schnelle, 2002). In low pH water transmission bundles, both the water conductivity increases and the water transmission becomes faster and the microorganism growth in the water decreases. For this reason, the pH value of the solution is adjusted to the desired degree by adding an acid to the solutions. Many compounds such as salicylic acid, succinic acid, citric acid are used in vase solutions (Kazaz et al., 2020).

Alaey et al. (2011) investigated the effects of preharvest and postharvest salicylic acid (SA) application on the vase life of cut roses. In the study, it was determined that both pre-harvest and post-harvest SA application increased the vase life, total solution uptake and relative fresh weight.

3. CONCLUSION

The criteria used to terminate the vase life are: fading of leaves, yellowing, petal shedding, bent-neck, flowers not blooming, etc. Knowing the factors that cause the flower to end its vase life is important in determining the substance(s) in the solution to be used to prolong the vase life. The effect, amount, application time and pH value of the substance to be used in the protective solution vary according to the type and variety of flower. After evaluating all these factors, the most suitable solution should be prepared.

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CHAPTER 11

PAST, CURRENT STATUS AND FUTURE OF SOME IMPORTANT POME FRUIT SPECIES IN TÜRKİYE

Assist. Prof. Dr. Halil İbrahim SAĞBAŞ[1]

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[1] Atatürk University, Faculty of Agriculture, Department of Horticulture, Erzurum, Türkiye. ORCID: 0000-0002-1402-309X, eposta: halil.sagbas@atauni.edu.tr

1. HISTORY OF FRUIT GROWING IN TÜRKİYE

According to the first records, human beings continued their economic life as hunter-gatherers with a nomadic lifestyle. The first people, who were busy with their essential needs such as hunting, nutrition, housing for a long time, considered the agricultural products as commercial commodities. After the discovery of plant production, people left the nomadic life, then moved to the established settled life. They laid the foundation of the concept of trade by exchanging their harvested products with other objects (Ercişli and Sağbaş, 2023). In the archaeological excavations, the first findings related to agriculture were found in the plains along the Nile River (in Mesopotamia), and the Indus Valley extending in the direction of Afghanistan, Pakistan and India (Diamond and Renfrew 1997; Diamond, 2002; Gerçekcioğlu, 2014). The region where wild forms together with the cultural cultivars existed spontaneously in nature are the homeland of the related plant species. Gene centers, which are called the homeland of plant species, are grouped under two groups as macro and micro. Macro gene centers are 1) India, 2) Central Asia, 3) Near East, 4) Mediterranean, 5) Ethiopia, 6) South America, 7) Mexico and Central America. Micro gene centers, which are the primary and secondary gene centers of most temperate climate fruit species in Türkiye, are: 1) Thrace and Aegean Region, 2) Southeastern Anatolia Region, 3) Region containing Samsun, Tokat and Amasya provinces, 4) Kayseri and its vicinity, 5) Erzurum-Ağrı (Altındal ve Akgün, 2015).

Compared to annual plants, fruit trees have continued their existence since the past by showing more tolerance to stress conditions, especially because of the strong root system of wild species, and being perennial plants. They have had an important place in the development of civilizations. The fruits have spread throughout the world through migrations and wars. Apple and pear were first cultivated in Central Asia, and brought to the European continent by Alexander the Great from Iran. Most fruit species from Mesopotamia spread to Egypt in the 20th century BC and to the eastern Mediterranean with the Jews just before their arrival in the 10th century BC. In 4500s BC, vineyards and olive groves became the symbol of that region in Ancient Greece (Anonymous, 2023a).

Anatolian lands have witnessed numerous migrations and tribes throughout the history of humanity, and thus have a cultural heritage.

According to the written sources belonging to the Hittites (1650-1250 BC), there was not much difference in the types of fruits grown in Anatolian lands. Temperate climate fruit species such as apple, apricot, pear, medlar, cherry and plum are frequently encountered in texts from the Hittite period (Koç, 2006; Ünar, 2019). While this has been the case in the past years, it is reported that a wide variety of vegetable and fruit production was carried out in the Ottoman State (1400-1899 period) in addition to grain production. Besides, a tithe tax is collected only from those grown and offered to the market. The temperate climate fruit species grown are as follows: Apple, pear, quince, hawthorn, medlar, rowanberry, almond, walnut, hazelnut, chestnut, plum, apricot, peach, cherry and apricot (Solak, 2008). In the Republican period (1923-1950) it started to become popular in public health and nutrition issues. Not only grain farming, but also the cultivation of many temperate climate fruit species (hazelnut, almond, walnut, peach, plum, apricot, cherry, sour cherry, apple and pear) was given importance. The production of all species of fresh and dried fruits in Anatolian lands suitable for their own ecology was supported. Due to the high economic value of some fruit species, Türkiye has been the leading exporter country for these species for years (Yurtoğlu, 2019).

2. THE CURRENT STATUS OF APPLE, PEAR AND QUINCE IN TÜRKİYE

2.1. Apple (*Malus domestica*)

Apple is taxonomically included in the *Rosacea* family, the *Pomoideae* subfamily, and the *Malus* genus. Apple is a cold temperate climate fruit specie, so it is extremely resistant to winter cold. Apple is one of the species of fruits that need cooling the most. The chilling requirement of the apple is between 100-2700 hours depending on the cultivars, provided that it is below + 7.2 °C (Ağaoğlu et al., 2010). During the dormant season, the trunk of the tree can withstand temperatures up to –35 °C, and its one-year-old shoots up to -20 °C (Karaca et al., 2023). Apple is a cold temperate climate plant. Therefore, even in the coldest regions of the world, summer apple cultivars can be grown.

Apple is a perennial woody fruit specie that continues its existence and is produced almost all over the world. Strong, semi-dwarf and dwarf rootstocks of the apple, which has a very high cultivar, have been developed by breeding studies. In this way, apple has become an ideal fruit specie that preferred in modern fruit growing (Özçağıran et al., 2004). While there are over 30 apple species and more than 6500 apple cultivars with different characteristics around the world, the number of apple cultivars in Türkiye is more than 500 (Coşkuner, 2018). In addition to fruit growing, the apple is also used in landscaping as an ornamental plant (Aslan, 2020). The visuals of the crab apple (*Malus floribunda*) is presented in Figure 1.

Figure 1. The use of apple *(Malus floribunda*) as an ornamental plant (Original)

Apple, which sugar content is a rich fruit, is used as a raw material for many food products (vinegar, fruit juice, wine, dried apple, jam, canned food, compote, etc.) in addition to being consumed fresh (Çetin, 2019).

Apple is one of the most important fruit species in terms of fruit growing, which ranks first in every parameter among temperate climate fruit types in the world, is the most produced after banana (124,978,578 tons) (Çetin, 2019). According to the United Nations Food and Agriculture Organization (FAO) data, world apple production is 93,144,358 tons. China is the leader in world table apple production with a production of 45,984,858 tons and an area of 2,092,512 ha. Although Türkiye ranks fourth in terms of area with 168,811 ha, it ranks second in terms of production amount (4,493,264 tons). While America is in the seventh place (117,441 ha) on the basis of area, it is the third largest country in the world in apple production. The graph of the most apple production amounts of the eight countries in the world is presented in Figure 2. (Anonymous, 2023b).

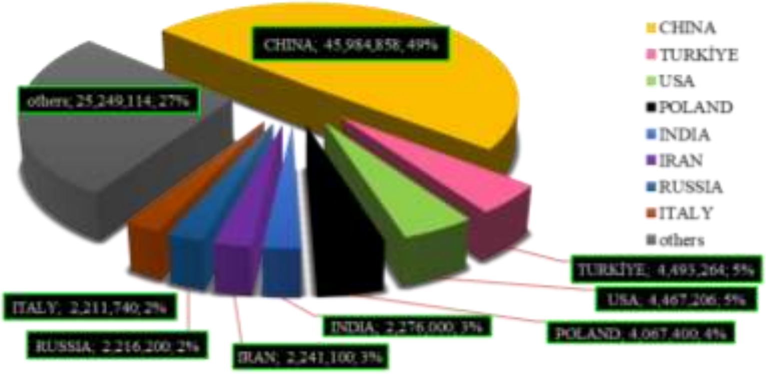

Figure 2. World apple production values according to FAO 2021 data (Anonymous, 2023b)

Due to the fact that four seasons are experienced and the altitude difference can reach very large values in the same province, temperate climate fruit species can be grown in almost all of Türkiye.

From this point of view, apple production has been made in all 81 provinces in Türkiye, according to Turkish Statistical Institute (TUİK) 2022 plant production statistics data. In Türkiye, it was produced 4,817,500 tons apples from 170,800 ha of agricultural land. While Isparta ranks third in terms of production area, it is at the top with 1,1230,000 tons of apple production. In terms of apple production, Karaman ranks second with 751,000 tons and Niğde ranks third with 543,000 tons. According to TUIK 2022 data, the graph of the five provinces with the highest apple production in Türkiye was given in Figure 3 (Anonymous, 2023c).

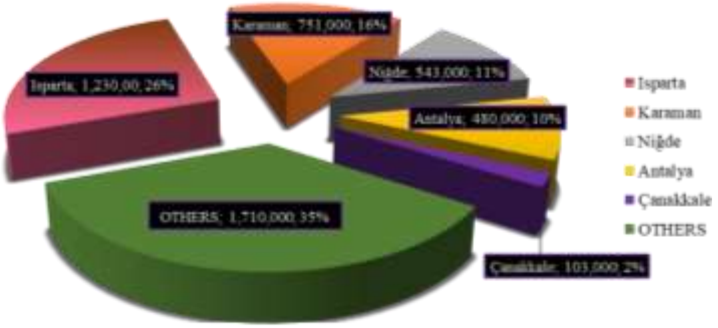

Figure 3. Apple production values in Türkiye according to TUIK 2022 data (Anonymous, 2023c)

2.2. Pear (*Pyrus communis*)

Pear is taxonomically included in the *Rosacea* family, the *Pomoideae* subfamily and the *Pyrus* genus. There are nearly 20 pear species in the world. In the 19th century BC, the name of the pear is mentioned in the first records as the grace of the Supreme Creator in the work of the Greek Writer Homer called "Odyssey". It is a specie of fruit that is resistant to dry conditions, and can be grown in warm temperate regions. Being resistant to drought has enabled it to be cultivated almost all over the world (Özçağıran et al., 2004). While pear is more resistant to high temperatures and drought than apples, it is more sensitive to low temperatures.

As in most fruit species, Türkiye is the gene center for pears (Akçay and Yücer, 2008). The most widely cultivated pear species around the world are European pears (*Pyrus communis*) and Asian pears (*Pyrus pyrifolia*). Commercially, European pear cultivars are more widely produced than Asian pear cultivars. Asian pears were cultivated about 3300 years ago, while European pears have been cultivated for over 2000 years. While Asian group pears are flat shaped, European group pears are long (pyriform) shaped. The visual of these two groups is presented in Figure 4 (Ekici and Yıldırım, 2017; Quinet and Wesel 2019).

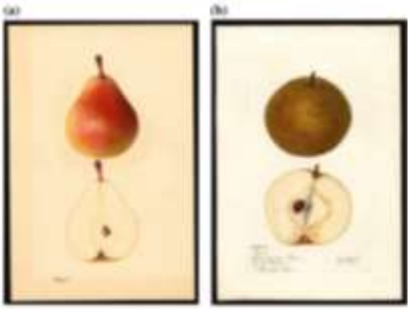

Figure 4. (a) European group and (b) Asian group pears (Quinet and Wesel 2019)

The chilling requirement of pear is generally lower than that of apples. It varies between 100-2300 hours depending on the cultivars, provided that it is below + 7.2 °C (Bolat et al., 2020).

Approximately 20% of the fresh fruit production in Türkiye consists of pome fruit species such as pear, apple and quince (Ekinci and Akçay, 2016). Problems encountered in cultivation, especially due to some diseases and pests seen in pears, cause more apples than pears to be produced. In particular, fire blight disease (*Erwinia amylovora*) causes great damage to pear production all over the world (Öztürk et al., 2011; Hunter, 2016). However, some positive developments in the cultivation technique of pear in recent years (the use of semi-dwarf and full-dwarf rootstocks) have a significant share in increasing the production amount (Ekinci and Akçay, 2016). Pear is the most produced fruit species after apple among temperate climate fruit species and mostly evaluated as table food (Özçağıran et al., 2004).

According to the United Nations Food and Agriculture Organization (FAO) data, world pear production is 25,658,713 tons on an area of 1,399,484 hectares. China, which is the third largest country in the world and the gene center of the pear, meets 74% of the world pear production with 18,798,144 tons from 986,479 hectares of agricultural land. The United States ranks second in world pear production with 636,390 tons of pear production from 16,876 ha of agricultural land. Argentina is in the third place in the world pear production with a production of 634,000 tons from an area of 24,802 ha. Türkiye produced 530,349 tons of pears from an area of 25,155 ha, taking its place in the fourth place in terms of production amount. The graph of the world pear production amount according to FAO 2021 data was given in Figure 5 (Anonymous, 2023b).

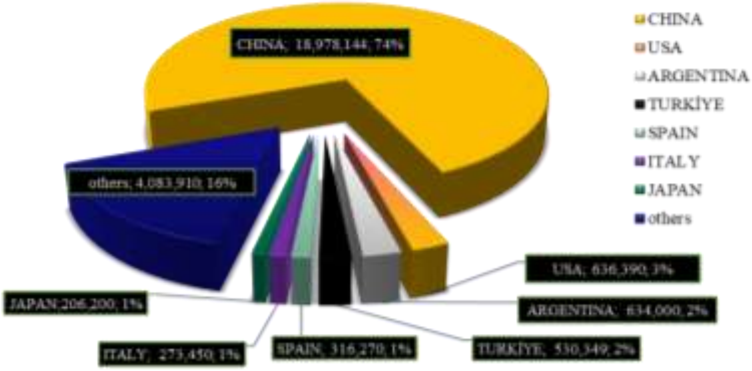

Figure 5. According to FAO 2021 data world pear production values (Anonymous, 2023b)

According to the 2022 data of the Turkish Statistical Institute, 551,086 tons of pears were produced from 237,063 decares of agricultural land in Türkiye. The total production area of the remaining provinces is 100,000 decares, except for the provinces that take the first seven places in pear production in Türkiye. In other words, the pears were produced in almost all provinces of Türkiye. Bursa has a 41% share in pear production with 225,798 tons. Antalya is in the second place (58,797 tons) both in terms of area and production amount. While Sakarya is in the fifth place (3,940 decares) in terms of pear production area, it is in the third place (16,442 tons) in terms of production. While Çanakkale is in the sixth place in terms of pear production area (3,909 decares), it is in the fourth place in terms of production (14,973 tons). The total area of the orchards where pear cultivation is carried out in Mersin is 5,822 decares and the production amount is 13,379 tons. According to TUIK 2022 data, the visual of the amount of pear production in Türkiye is presented in Figure 6 (Anonymous, 2023c).

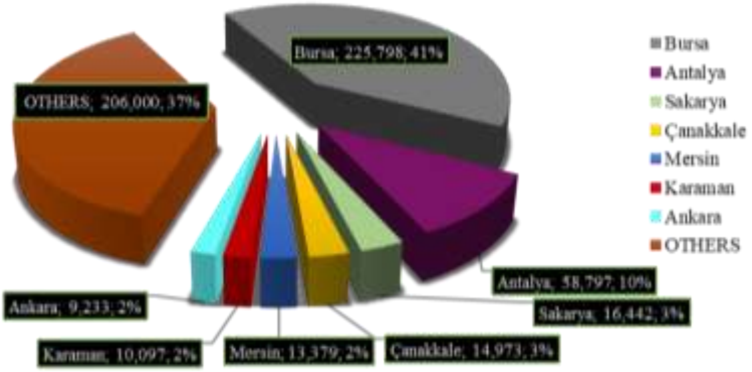

Figure 6. According to TUIK 2022 data pear production values in Türkiye (Anonymous, 2023c)

2.3. Quince (*Cydonia oblonga* Mill.)

Quince is represented by *Cydonia oblonga* Mill. which only one species in the *Cydonia* genus of the *Rosaceae* family. The fruits are pear (pyriformis) or apple (maliformis) shaped, but the most common is the pear-shaped one (Abdollahi, 2019). The visual of fruit shapes of different pear cultivars is presented in Figure 7 (Hussain et al., 2021).

Figure 7. Some quince varieties with different fruit forms (Hussain et al., 2021)

The fruits of quince grown in subtropical and temperate climatic conditions are juicy and tender. Therefore, they can be consumed immediately after harvest without the need for any processing. But the fruits of quince grown in cool climates are suffocating, hard and sandy at harvest time. This situation, which is a problem in table consumption, can be eliminated by keeping the quince at 20 °C for a few weeks (Rop et al., 2011).

It is known that the gene center of quince is Middle East and Central Asia, especially Türkiye. Quince is associated with happiness, love and fertility in Greek mythology. It is thought that the name *Cydonia* was inspired by Kydonia, an ancient city in Greece (Mir et al., 2016). Quince can adapt to a wide variety of climatic conditions and soils, but its hairy root system makes it susceptible to drought or low water availability. Therefore, irrigation may be required during the vegetation period (Sastri, 1950). Its fruits are green in its raw state, and its exocarp is covered with dense gray hairs. This condition of hairiness continues to decrease as the fruit matures (Sharma et al., 2011). At full yield period, a quince tree can produce fruit between 10-60 kg, while a single fruit can reach a weight of 4.50 kg (10 lb) (Ahmad et al., 2004). Although quince is known for its strong aroma and flavor, it is not preferred much in table consumption due to its very hard structure, astringency and tartness (Rasheed et al., 2018). However, quince is one of the fruit species with high pectin content (1.8 g/100 g FW). This situation enables the production of jam, jelly, marmalade, pudding, candy (Sharma et al., 2011), syrup and pasteurized fruit juice by processing the fruit in the food industry (Wilson, 1999). In addition, quince is also used in the production of low-alcohol beverages in Balkan countries (Radovic et al., 2019). Quince also has an important place in Balkan culture. When a child is born in the Balkans, a quince tree is planted to represent the continuation of the generation, life and love (Sağbaş ve Ercişli, 2023). It is a fact known by everyone that the leaves and fruits of quince are consumed by boiling, it is good for colds.

Quince is a specie of pome fruit that is produced less amount both in the world and in our Türkiye compared to apples and pears. According to FAO 2021 data, 697,562 tons of quince was produced from 75,894 hectares of agricultural land in the world. Although Türkiye ranks third in the world (7,908 ha) in terms of quince production area, it is the leader in terms of production amount with 192,012 tons. Although China ranks first with 32,477 ha in terms of quince production area, it ranks second in terms of production amount (111,377 tons). Then, the countries that have a say in quince production are Uzbekistan (third) with 97,536 tons, Azerbaijan is the fifth with 42,065 tons (Anonymous, 2023b).

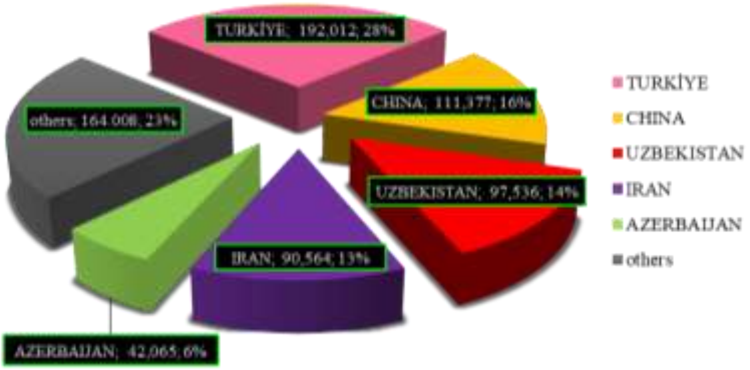

Figure 8. According to FAO 2021 data world quince production values (Anonymous, 2023b)

According to TUIK 2022 data, 197,503 tons of quince was produced from 80,647 decares of agricultural land in Türkiye. Sakarya occupies 33% (26,991 da) of quince production orchards and 52% (102,413 tons) of production amount, which is the leader in Türkiye. In Sakarya province, the places with the highest quince production are Geyve district (58.148 tons/12.505 da) and Pamukova district (40.401 tons/13.100 da). Geyve district is the leader in this field in Türkiye by producing even more than Azerbaijan, which ranks fifth in quince production in the world. Therefore, Geyve, as a district, has produced more than a country that has a say in quince production, and it has demonstrated its importance. The provinces with the highest quince production area in Türkiye are: Sakarya, Denizli, Bursa, Bilecik, İzmir, Isparta, Çanakkale and Amasya. In terms of, provinces with the largest share quince production amount are Sakarya (102,413 tons), Bursa (15,118 tons), Denizli (8,556 tons), Çanakkale (7,679 tons), Bilecik (6,591 tons), Izmir (5,127), Isparta (4,486 tons). tons) and Amasya (4,074 tons), respectively. According to TUIK 2022 data, the graph of the production amounts of the provinces that have a say in Türkiye's quince production is given in Figure 9 (Anonymous, 2023c).

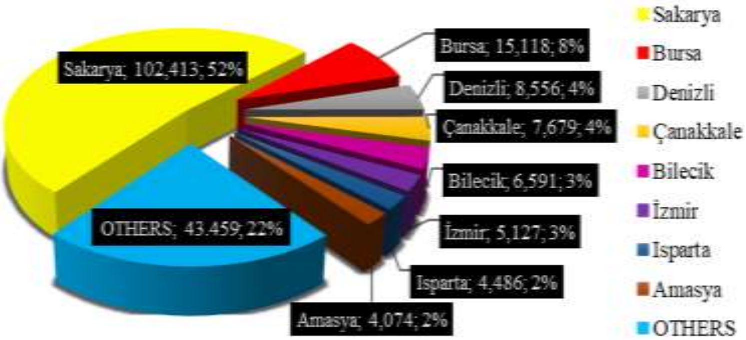

Figure 9. Türkiye quince production values according to TUIK 2022 data (Anonymous, 2023c)

3. THE FUTURE OF APPLE, PEAR AND QUINCE IN TÜRKİYE

The increasing awareness of fruit consumption, which is one of the most important items of healthy nutrition, and the spread of value-added food producing establishments with the effect of rapidly developing technology have enabled fruit growing not only to be a branch of agriculture, but also to turn into a systematic agricultural production industry. The use of materials and methods resistant to different biotic and abiotic stress conditions, with the effect of technological developments in cultivation techniques, has increased the diversity of species and varieties in fruit growing. In addition, the biggest proof of this is to see that fruit species that cannot be grown under normal conditions in a region where continental climatic conditions are experienced can be grown in time due to global warming, which has made its impact felt a lot in recent years. When examined from this point of view, the presence of dwarf, semi-dwarf and strong rootstocks and genetic diversity in species such as apple, pear and quince, show that these species have high cultivated potential.

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