



# Agricultural Practices and Sustainable Management in Türkiye

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IKSAD  
Publishing House

# AGRICULTURAL PRACTICES AND SUSTAINABLE MANAGEMENT IN TÜRKİYE

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Iksad Publications – 2022©

**ISBN: 978-625-8213-37-9**

Cover Design: Yusuf SOLMAZ, Korkmaz BELLİTÜRK

September / 2022

Ankara / Türkiye

Size = 16x24 cm

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## PREFACE

In general, "sustainability" is based on the principle that we must meet our needs without compromising and hindering the ability of future generations to meet their own needs. Nowadays we are facing problems such as: Hunger in poor countries, climate change, rising costs of food, fuel and transportation, pollution from pesticide use, adaptation and resistance to pests, loss of soil fertility and organic carbon, soil erosion, biodiversity reduction, desertification, etc. Which pose a serious threat to the continuity of life on earth. Despite unprecedented advances in science that allow us to visit other planets and discover subatomic particles, the earth's most important food-related issues clearly show that conventional agriculture is not yielding adequate results for feeding humans and preserving ecosystems. Sustainable agriculture is an alternative to solving the basic and applied issues related to food production in an environmentally friendly way. While conventional agriculture aims at almost only productivity and profitability, sustainable agriculture integrates biological, chemical, physical, ecological, economic and social sciences in a comprehensive way to develop new agricultural practices that are safe and do not degrade our environment. To address current issues and practices used in relation to sustainable agriculture in Turkey and to promote discussions and cooperation on this issue, we have provided this book with a summary of papers that provide different solutions and ideas regarding the practices of development of sustainable agriculture in our country.

Sincerely Yours, September, 2022

**Assoc. Prof. Dr. Korkmaz BELLİTÜRK**

**Dr. Yusuf SOLMAZ**



## CHAPTER 1

### NEW GENERATION FERTILIZERS IN AGRICULTURAL INPUTS: THE CASE OF VERMICOMPOST

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## INTRODUCTION

The agricultural waste may be managed in a variety of ways: Organic wastes are broken down into simpler forms during the composting process by microorganisms, mostly bacteria and fungus. The carbon in the waste serves as a fuel source for the bacteria. The breakdown of the original ingredients into a much more homogenous product that may be utilised as a soil amendment happens as a consequence of the degradation of the nitrogen-containing elements (Uyizeye et al., 2019). Many undesirable species, including weed seeds and diseases, are killed by the heat produced during the process. Composting has many benefits, including a decrease in waste volume, the removal of pests that have been heat-killed, and the creation of useful and marketable materials (Ayilara et al., 2020). By raising the amount of organic carbon in the soil, compost application may enhance soil qualities that are desperately in need of rejuvenation. Additionally, compost acts as a soil intervention to enhance soil tilth, water holding capacity, water penetration rate, and structure (Coelho et al., 2019).

Organic matter is known to enhance the health of the soil and the availability of nutrients for plants (Lasaridi et al., 2018). Although the organic wastes might be applied directly to the soil in their raw state, this is often not ideal for the health of the soil. According to custom, organic wastes are employed in t/ha to increase crop output, whether they have been composted or not (Aruna et al., 2018; Lasaridi et al., 2018). Because of this, the availability of organic wastes in enormous bulk quantities to be applied at several t/ha might be a limiting issue in its scope and may also not be financially viable. Compost technology as a solid waste management technique has lately attracted increased attention. A clever treatment of decomposed material may not only lower compost application rates but also aid in producing a product with the necessary properties. The enrichment or

mixing of the compost with certain particular nutrients (organic fertiliser) and plant growth-promoting rhizobacteria is an innovative way to turn decomposed waste into a value-added product (bio-fertilizer). Such value-added soil amendments have been discovered by Ventorino et al. (2019) to have extremely beneficial impacts on soil health and maize yield.

Vermicomposting is the alternative method for dealing with agricultural waste (Bellitürk, 2016; Bellitürk, 2018). The physical and biological characteristics of the raw material are changed by the earthworms' strong interactions with various microbes and animals of the decomposer community via vermicomposting. Part of the nutrients in organic materials are changed to make them more accessible. It is a finely structured substance that resembles peat and has high porosity, strong aeration, drainage, microbial activity, high water-holding capacity, rich nutritional status, and good buffering capacity; optimal physiochemical characteristics crucial for plant development and soil fertility (Pathma and Natarajan, 2012). The abundance of hormones and enzymes produced during the passage of organic materials through the earthworm's intestines may promote plant development while suppressing plant diseases and ensuring the production of hygienically sound goods. Vermicomposting produces useful biofertilizer up to two to five times quicker than thermophilic composting (Atiyeh et al., 2000). Various organic wastes, including sewage sludge (Benitez et al., 1999), paper-mill industry sludge (Butt, 1993), pig waste (Reeh, 1992), crop residues (Bansal and Kapoor, 2000), cow dung (Aira et al., 2011), biogas slurry, rice straw (Mitchell, 1997), etc., have been tested as feed material for earthworm species. Plant growth-promoting rhizobacteria, often known as beneficial rhizobacteria or PGPR, influence plant development either directly or indirectly via a variety of methods of action (Korir et al., 2017; Egamberdieva et al., 2017). According to Ahmad et al. (2020), certain PGPR work as sinks for 1-

amonocyclopropene-1-carboxylate (ACC), the direct precursor of ethylene in higher plants, by bringing down the amount of endogenous ethylene in the plant. PGPR with ACC- deaminase characteristic are attractive candidates for bio-fertilizer formulation since they often provide extremely consistent results in terms of boosting plant growth and output (Korir et al., 2017).

Approaches and technology that enhance soil health and the environment over the long term must be urgently modified. This strategy has contradictory implications, such as: 1). Creation of a helpful soil amendment for agricultural crops and soil health. 2). The amount of environmental contamination brought on by the enormous amounts of organic waste might be reduced.

## **EARTHWORMS AND RAW MATERIAL FOR VERMICOMPOSTING**

There are more than 3000 different species of earthworms in soil, but only around 8 to 10 of them are good for making vermicompost (Cook and Linden, 1996). Epigeic species of earthworms, such *Eisenia fetida* and *Eudrilus eugeniae*, are the finest kinds for vermiculture and vermicomposting (Bansal and Kapoor, 2000; Dominguez and Edwards, 2004). As opposed to worm species that feed on plain soil, they prefer to reside on the soil's top surface and consume organic material like vegetable waste, compost, and organic bedding in order to generate rich material. In comparison to other species, these animals consume up to half of their body weight each day, and by breaking down and decomposing natural remnants, they generate high-quality organic compost. Additionally, the aforementioned earthworm species are resilient to changes in humidity and temperature. These species are always active throughout the year, break down organic material quickly, and quickly create vermicompost. Red worms of other species, such as *Lumbricus*

*rubellus*, *Perionyx sansibaricus*, *Perionyx excavatus*, *Eisenia andreii*, etc., might be utilized to produce vermicompost with similar results (Dominguez and Edwards, 2004). Any material that decomposes quickly is used for vermicomposting, including cow dung, weeds, fruits and vegetable leaves, animal roughage, agricultural leftovers, and municipal trash that are organic in nature (Kiehl, 2001). The organic waste that an earthworm consumes is physically broken down in the gizzard before being exposed to several enzymes, including chitinase, cellulose, lipase, amylase protease, and others, that are released into the lumen by the gut wall and related bacteria. These enzymes convert intricate biomolecules into more basic ones. Mucus secreted by the gut wall provides vermicompost stability. Earthworms only absorb 5-10% of the material they need for development; the remainder is expelled as casting.

### **VERMICOMPOST AS BIOFERTILIZER**

The use of biofertilizers is now recognized to have a number of positive effects on soil, including the solubilization of important minerals, the absorption of nutrients, the provision of micronutrients in forms that are more readily absorbed by plants, and participation in biological nitrogen fixation. *Azospirillum*, *Azotobacter*, phosphor-bacteria, *Rhizobia*, and cyanobacteria are among the plant growth-promoting microorganisms (PGPMs) that belong to this category of microbes. The PGPMs may impart beneficial effects on the growth and yield characteristics of a variety of cultivable crops in various locations of the globe (Mahanty et al., 2017). Rhizosphere bacteria increase the availability of nutrients, inhibit the development of plant diseases, or produce hormones like auxins to encourage plant growth (Kumar et al., 2017). Utilizing conventional culture medium or main source augmented media, biofertilizers are mass produced in a lab for agricultural use (Backer et al., 2018; Mahajan and Gupta, 2009; Meena et al., 2020). According to a recent

study by Vyas and Gulati (2009), the use of vermicast as a carrier material may boost the survival rate of biofertilizer organisms for up to a year.

Vermicomposting dramatically increases microbial diversity and activity, and the resulting material may contain plant growth regulators produced as a result of interactions between microorganisms and earthworms, which may have a significant positive impact on plant growth, flowering, and yields (Schütz et al., 2018). In order to promote plant growth and production, microbial inoculants such biofertilizers might be added or enhanced. There are no studies on the microbial enrichment of vermicompost that take into account the quantity of inoculum needed, the time of inoculation, the survival rate of inoculated microorganisms in vermicompost during storage, or the relationship between the total microbial population and that of inoculated microorganisms. Therefore, the current study was carried out to assess the survival rate of *A. brasilense* and *R. leguminosarum* in enriched vermicompost in relation to the total microbial population and to optimize the inoculum level and time of inoculation of biofertilizers in enrichment process of vermicompost (Schütz et al., 2018).

### **MECHANISM OF ZINC SOLUBILIZING, PHOSPHORUS SOLUBILIZING AND CELLULYTIC MICROBES AS BIO-FERTILIZER**

According to the definition given by the term "bio-fertilizer," it is "a product that comprises live microflora that integrated to plant surfaces, seed or in soil, colonize within or the rhizosphere and promote the development by giving primary nutrients to plants" (Vessey, 2003). A further modification to the definition was made by Dineshkumar et al. (2018) who defined it as "the liquid-based products comprising dormant or living microbiota (fungi, bacteria, actinomycetes) individually or in association, which incorporate in

solubilizing soil nutrients atmospheric nitrogen (N) fixation and additionally secretion growth promoting substances for increasing crop growth and yield attributes".

The microorganisms that live in biofertilizers use a variety of techniques to benefit agricultural plants. They may be proficient at all the processes—nitrogen fixation, phosphate solubilization, zinc solubilization, cellulose degradation (Table 1, Figure 1-3) and plant growth promotion—or they may combine all (Mahanty et al., 2017; Zandi and Basu, 2016; Bhardwaj et al., 2014; Ritika and Utpal, 2014). Through the biological nitrogen fixation (BNF) process, biofertilizers may fix atmospheric N<sub>2</sub>, as well as solubilize plant-necessary minerals like phosphate, zinc, and potassium, and secrete compounds that encourage plant development, such as a variety of hormones (Borkar, 2015; Kumar et al., 2018). Additionally, biofertilizers may grow and take part in nutrient cycling when used as seed or soil inoculants, which aids in crop development for sustainable farming (Itelima et al., 2018; Singh et al., 2011). The microbial inoculants are superior than their chemical counterparts in a number of ways (Backer et al., 2018; Mahajan and Gupta, 2009; Meena et al., 2020). They are sustainable, ethical sources of the nutrients needed to support soil biology and health (Raklami et al., 2019, Bhardwaj et al., 2014, Sun et al., 2020). Additionally, they fight abiotic stressors and show antagonistic action against a number of agricultural diseases (Ilangumaran and Smith, 2017; Timmusk et al., 2015; Bharti et al., 2016; Sharma et al., 2016; Timmusk et al., 2014). Based on their capacity to draw nutrients from the soil, fix atmospheric N<sub>2</sub>, promote the solubilization of nutrients, and function as biocontrol agents, a variety of microbial taxa have been employed commercially as effective biofertilizers (Schütz et al., 2018).

**Table 1.** Mechanism of Zn solubilizing bacteria (ZSB).

<b>Bacteria</b>	<b>Mechanism of solubilization</b>	<b>Reference</b>
<i>Bacillus cereus</i>	Organic acid production	(Kumar et al., 2017)
<i>Pseudomonas</i> sp. and <i>Bacillus</i> sp.	Organic acid production	(Saravanan et al., 2004)
<i>Pseudomonas fluorescens</i>	Production of organic acid	(Di Simine et al., 1998)
<b>Mechanism of Zn solubilizing bacteria (ZSB)</b>		
<i>Pseudomonas corrugata</i> NRRLB-30409	Production of 2-Ketogluconic acid	(Trivedi and Sa, 2008)
<i>Bacillus cereus</i> , <i>Bacillus subtilis</i> , <i>Paenibacillus</i> sp	Production of Oxalic acid, malic acid, formic acid, acetic acid, tartaric acid and gluconic acid	(Chawngthu et al., 2020)
<i>Pseudomonas trivalis</i> BIHB 769	Production of Gluconic acid, ketogluconic acid, lactic acid, fumaric acid, malic acid and succinic acid	(Vyas and Gulati, 2009)
<i>Pseudomonas poae</i> BIHB 751	Gluconic acid, ketogluconic acid, citric acid and malic acid	(Vyas and Gulati, 2009)

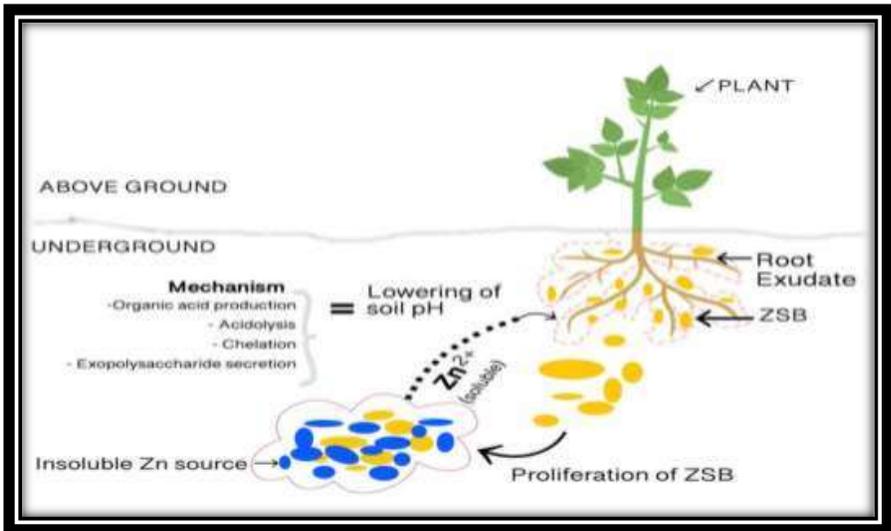


Figure 1. Zn solubilizing bacteria mechanism (Rani et al., 2020).

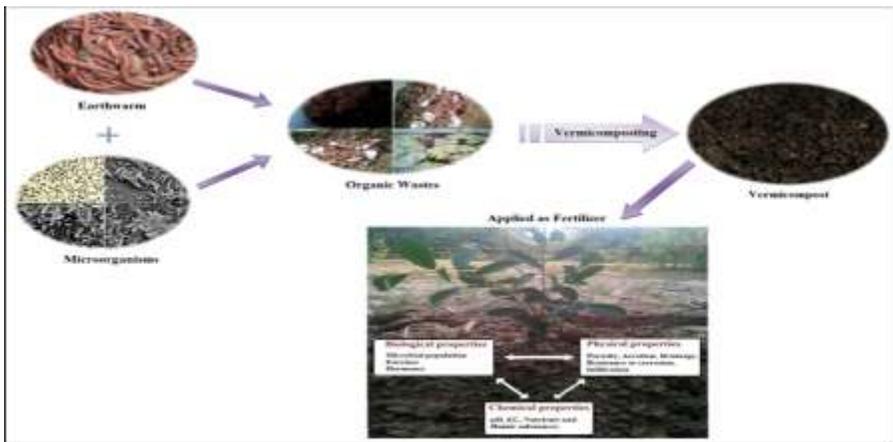
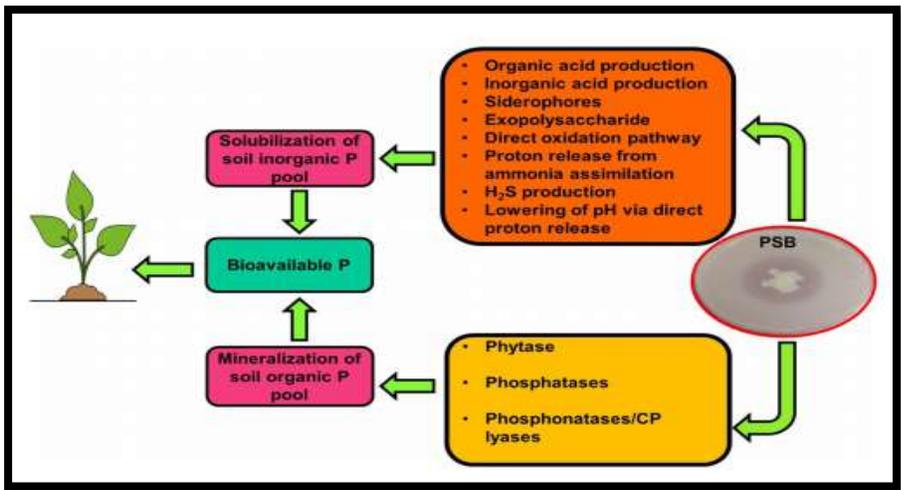


Figure 2. Cellulolytic bacteria mechanism (Sharma and Garg., 2017).



**Figure 3.** Phosphorous solubilizing bacteria mechanism (Rawat et al., 2020).

Researchers' focus has changed away from using chemical fertilizers and pesticides in favor of organic fertilizers like vermicompost, which may boost crop yield and protect it from destructive pests without harming the environment (Joshi et al., 2014). Vermicompost is produced when earthworms and other microorganisms speed up the biological breakdown of organic wastes. By passing the organic waste through a gizzard that grinds it into smaller pieces, earthworms absorb the trash and get their nutrition from the microorganisms that colonize it. The procedure increases the rates of organic matter deterioration, modifies the material's physical and chemical characteristics, and produces a humification effect in which the unstable organic matter is completely oxidized and stabilized (Orozco et al., 1996). The earthworms' fragmentation of the parent organic components and the microorganisms' colonization of the sand considerably humify the final result, which is known as vermicompost (Edwards, 1998).

Bacteria, actinomycetes, fungus, and cellulose-degrading bacteria are abundant in vermicompost (Werner and Cuevas, 1996). According to Tomati

et al. (1983), earthworm castings, which are produced following sludge digestion, are abundant in microorganisms, particularly bacteria. The microorganisms connected to vermicompost and those in conventional composts were compared by Nair et al. (1997). In comparison to ordinary composts, the vermicompost contained substantially bigger populations of bacteria ( $5.7 \times 10^7$ ), fungus ( $22.7 \times 10^4$ ), and actinomycetes ( $17.7 \times 10^6$ ). Vermicompost is made up of finely split mature peat-like materials with a high porosity, aeration, drainage, and water-holding capacity. It is stabilized by interactions between earthworms and microorganisms in a non-thermophilic process (Edwards and Burrows, 1988).

The majority of plant nutrients found in vermicompost are present in forms that plants may use, including nitrate, phosphates, exchangeable calcium, and soluble potassium (Orozco, 1996). Compared to commercial plant growth media, vermicompost made from animal waste typically contains more mineral elements, and many of these elements have been changed to forms that make them easier for plants to absorb, like nitrates, exchangeable phosphorus, and soluble potassium, calcium, and magnesium (Edwards and Burrows, 1988). Similar to this, Orozco et al. (1996) found that following processing by *Eisenia fetida*, coffee pulp improved the availability of nutrients including phosphate, calcium, and magnesium.

Vermicompost was characterized by Joshi et al. (2014) as a superior organic fertilizer and a biocontrol agent via a variety of research, as well as the reasons why it is more environmentally friendly than chemical fertilizers. As a result, the goal of this research is to evaluate how plants react when vermicompost is applied.

## **CHARACTERISTICS OF VERMICOMPOST**

Vermicompost was described by Adhikary (2012) as the worm excretions that may enhance the nutritional content and overall health of the soil. Vermicomposting is the process by which all kinds of biodegradable wastes, including those from farms, kitchens, markets, agro-based companies, animals, etc., are transformed into nutrient-rich vermicompost as they travel through worm guts. Vermicompost, which is comprised of compost that has been digested, is a great soil enhancer. Compared to conventional composts, it has a greater nutritional value. This is a result of the earthworms' higher humification and mineralization rates (Albanell et al., 1988). Additionally, it greatly improves soil fertility by enhancing the physical features of the soil. This is due to the high porosity, aeration, drainage, and water-holding capacity of vermicompost (Edwards and Burrows, 1988). Vermicompost also contains nutrients including nitrates, phosphates, exchangeable calcium, and soluble potassium in forms that plants may use (Orozco et al., 1996). Additionally, the author noted that vermicompost improved N availability and that it also increased the availability of C, P, K, Ca, and Mg in the castings (Orozco et al., 1996).

## **VERMICOMPOSTING PROCESS**

Earthworms and microbes work together in the breakdown process known as vermicomposting (Aira et al., 2000). Earthworms in particular are employed to speed up the waste conversion process and create superior products (Adhikary, 2012).

## **NUTRIENTS IN VERMICOMPOST**

Vermicompost, which is comprised of compost that has been digested, is a great soil enhancer. Worm castings are regarded as a better value product since they contain much more nutrients and microbial life (Adhikary, 2012).

Up to five times as many plant-available nutrients are present in worm castings than in typical potting soil blends. Castings were chemically analyzed (Ruz-Jerez et al., 1992), and it was discovered that they contain five times as much nitrogen, seven times as much potash, and one and a half times as much calcium as 15 cm of healthy top soil. Additionally, compared to other potting mixes, the nutritional life is up to 6 times longer. According to reports, phosphorus is changed into a form that plants may use during transit through worms' guts (Reinecke et al., 1992). Typically, phosphorus is seen as a limiting ingredient for plant development. As a result, any method that considerably increases the availability of phosphorus via plants and organic matter will be crucial for agriculture. The typical potting soil mixtures sold on the market are often sterile and devoid of any microbial life, the microbial and nutritional mix.

### **EFFECT OF VERMICOMPOST ON PLANTS**

Vermicompost includes the nutrients and other necessary elements like phosphorus and potassium, which greatly boost plant development (Fernandez et al., 2010). Numerous field studies have shown the beneficial results of even modest vermicompost treatment rates to crops (Norman and Clive, 2005). Vermicompost has successfully boosted the output of tomatoes and okra (Suthar, 2010). Similarly, Sallaku et al. (2009) discovered that the application of vermicompost considerably increased the relative growth rate of cucumber (*Cucumis sativus*) seedlings. Numerous studies also shown that using vermicompost together with the suggested inorganic fertilizer might boost crop output in the majority of cases. Vermicompost and half of the recommended inorganic fertilizers were applied together, increasing tomato output (Kolte et al., 1999). Vermicompost applied to field plots at rates of 20 t/ha and 10 t/ha and at rates of 8 t/ha and 5 t/ha, respectively, significantly increased the growth and yields of field tomatoes and peppers when compared

to those receiving equivalent amounts of inorganic fertilizers, according to Arancon et al. (2002). According to Ushakumari et al. (1999), the application of vermicompost at a rate of 12 t/ha together with 100% or 75% of the advised fertilizers boosted okra yields. Similar to this, Athani et al. (1999) show that vermicompost applied at rates of 2 kg/plant together with 75 percent of the recommended number of inorganic fertilizers enhanced banana shoot output. Tomato yields were enhanced to a level comparable to that of tomatoes grown in soil treated with 4 t/ha vermicompost and half of the prescribed rates of inorganic fertilizers by using a reduced application rate of 2 t/ha vermicompost together with the appropriate quantities of inorganic fertilizers (Patil et al., 1998). After supplementing the soils with 2.5 t/ha of vermicompost and 75% of the prescribed inorganic fertilizers, potatoes achieved the highest commercial yields (Mrinal et al., 1998). Sunflowers produced the most after soil treatments with 50% of the recommended inorganic fertilizers application rates and 5t/ha or 10t/ha of vermicompost (Devi et al., 1998). Peas produced more and yielded more when soils were amended with farm manure applied at a rate of 10t/ha, vermicompost made from it, and the full recommended application rate of inorganic fertilizers (Ramachandra et al., 1998). Sugarcane yields were found to have risen by Zende et al. (1998) after soils were amended with vermicompost at rates of 5t/ha together with 100 percent of the recommended application rate of inorganic fertilizers. Vermicompost-applied wheat crops have improved in production and growth, according to studies. Wheat yield increased by more than 40% to the use of vermicompost fertilizers (Palanisamy, 1996). Vermicompost has been shown to have positive agronomic effects on rice crops (*Oryza sativa*), including increased populations of nitrogen fixers, actinomycetes, and mycorrhizal fungi that promote improved crop development and nutrient absorption (Kale et al., 1992).

## **PHYSICO-CHEMICAL CHANGES IN SOILS IN RESPONSE TO VERMICOMPOST APPLICATIONS**

There are a number of reasons why crops grown in greenhouse potting medium or in field soil that has been replaced or altered with vermicompost have shown benefits in growth and yield. Vermicompost helps to enhance the physical, chemical, and biological properties of planting medium and field soils, which in turn helps plants develop more successfully. The measured pH changes, however, were different from those reported by Tyler et al. (1993), who found increases in substrate pH in response to progressively higher additions of composted turkey litter to a medium for plant containers. As salt content rose, it caused a linear rise in electrical conductivity in pig dung vermicompost (Atiyeh et al., 2001a, b).

Vermicompost was added to soils, which resulted in a considerable rise in the amounts of N, P, and K in the soil (Venkatesh et al., 1998, Sreenivas et al., 2000). The amounts of total extractable N, microbial biomass N, and dissolved organic N in soils planted with strawberries were statistically comparable across all treatments at the end of the strawberry growth cycle, but soils treated with vermicompost had higher levels of orthophosphates than soils treated with inorganic fertilizers. In response to vermicompost treatments, Masciandaro et al. (1997) found that soils had higher levels of organic carbon, better pH, reduced bulk density, increased microbial populations, and enhanced dehydrogenase activity.

## **PLANT GROWTH REGULATOR PRODUCTION IN VERMICOMPOST**

Numerous studies have shown that microorganisms, such as bacteria, fungi, yeasts, actinomycetes, and algae, are capable of generating significant amounts of plant growth hormones and plant growth regulators (PGRs),

including auxins, gibberellins, cytokinins, ethylene, and abscisic acid (Arshad and Frankenberger, 1993). The creation of plant growth-regulating compounds by mixed microbial populations in soil has been extensively studied, but their availability to plants, persistence, and destiny in soils, as well as the reliable documentation of their effects on plant development, have received far less attention (Arshad and Frankenberger, 1993).

PGRs may be absorbed by plants from soil in sufficient amounts to affect plant development, according to Norman and Clive's (2005) review. Auxins generated by *Azospirillum brasilense* were demonstrated to have an impact on the development of graminaceous plants (Kucey, 1988). Gibberellins may also affect the development and growth of plants (Arshad and Frankenberger, 1993). *Arthrobacter* and *Bacillus* spp. synthesis of cytokinin in soil has been linked to seedlings' increased vigour (Jagnow, 1987). It is likely that vermicompost might be a reliable source of plant growth regulators created by interactions between microorganisms and earthworms since vermicomposting improves microbial variety and activity. This could considerably improve plant growth, blooming, and yields. Nielson (1965), who extracted indole compounds from earthworms and found increases in pea growth owing to the earthworm extracts, proved the existence of plant growth regulating chemicals in the tissues of *Aporrectodea caliginosa*, *Lumbricus rubellus*, and *Eisenia fetida*.

It has been noted that vermicompost made from animal manure, sewage sludges, or paper-mill sludges contains significant levels of humic compounds (Senesi et al., 1992; Garcia et al., 1995; Masciandaro et al., 1997; Elvira et al., 1998). Studies on the impact of humic compounds on plant development have consistently shown beneficial growth benefits when appropriate mineral feeding is present (Chen and Aviad, 1990).

## SUPPRESSION EFFECT OF VERMICOMPOST

According to Shakir and Mikhal (2004), either general or targeted suppression techniques may dramatically reduce the prevalence of plant diseases like *Pythium*, *Rhizoctonia*, and *Verticillium*. Additionally, they showed that adding vermicompost to soils had a major impact on the trophic structure of nematode populations, drastically reducing populations of plant parasite species. Vermicompost may reduce the quantity of feeding and damage caused by sucking pests like aphids and mealy bugs and chewing pests like caterpillars, according to trials conducted in greenhouses.

According to studies, organic amendments help prevent plant illnesses (Lazarovitis et al., 2001; Fikre et al., 2001). Chanyasak et al. (1983) observed that the use of vermicompost prevented the growth of *Plasmodiophara brassicae*, *Phytophthora nicotianae* (late blight of the tomato), and *Fusarium lycopersici* (fusarium wilt of the tomato). Szczech (2002) observed that vermicompost was effective in *Phytophthora nicotianae* and *Fusarium lycopersici* suppression on tomatoes. By adding vermicompost to the growing medium, Rodriguez et al. (2000) showed that gerbera plant diseases such *Rhizoctonia solani*, *Phytophthora drechsleri*, and *Flisarium oxysponlm* are generally suppressed. Aqueous extracts of vermicomposts were shown to suppress the mycelial development of *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Corticium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum*, according to research by Nakasone et al. (1999). Some important information regarding the superior properties of vermicompost is given in Figure 4 below.



**Figure 4.** Some important superior of vermicompost.

## EFFECT OF VERMICOMPOST ON PLANT DISEASES

Numerous studies have shown that vermicompost is beneficial in preventing a variety of plant diseases (Chaoui, 2002; Arancon, 2002). Vermicompost is helpful in protecting plants from a variety of illnesses, according to several research (Moradi et al., 2014). The resident microbial population, of which fungi play a very significant part, is the active component engaged in the biodegradation and conversion process during composting in vermicomposting (Wiegant, 1992).

## SUMMARY AND CONCLUSIONS

It has long been understood in agriculture that using organic matter, such as animal manures, human waste, food wastes, yard wastes, sewage sludge, and composts, is helpful for plant development and production as well as the preservation of soil fertility. Vermicomposting, one of the newest methods for applying organic amendments to farms, has been shown to be a successful method for raising soil fertility, crop yields, and soil structure.

Even at a very low rate, vermicompost may boost the development, blooming, and yields of agricultural and ornamental crops. Vermicompost's effects on plants are due to a variety of growth-regulating substances, including humic acids and plant growth hormones, in addition to the high quality of mineral nutrition it provides. Vermicomposting also improves soil quality by boosting microbial activity and biomass, which are essential for nutrient cycling, the generation of plant growth regulators, and the defence of plants against insect assaults and soil-borne diseases.

### **ACKNOWLEDGEMENTS**

The authors gratefully acknowledge HEC for providing financial support to carry out this research work under HEC projects [“NRPU-HEC project no. 7527/Punjab/NRPU/R&D/HEC/2017\_ Vermicomposting: A resourceful organic fertilizer to improve agriculture production and soil health and Second project “Vermicomposting: An Agricultural Waste Management Technology”, Pak-Turk Researchers Mobility Grant Program Phase- II, vide letter No. (Ph- II-MG-9)/ PAKTURK/ R&D/HEC/2018”]. The authors are also thankful to Punjab Agriculture Research Board who financially supported the project (Project# 18-550) entitled with “Developing Agricultural Waste Management System to Produce Different Kinds of Organic Fertilizers for Sustainable Agriculture”

### **AUTHOR’S CONTRIBUTION**

KB, Z.A. and AA: Conceived and designed the experiment, performed the experiment, analyzed the data, contributed reagents/ materials/ analysis tools, wrote the paper.

### **CONFLICT OF INTERESTS**

The authors have declared no conflict of interest.

## REFERENCES

- Adhikary, S. (2012). Vermicompost the story of organic gold: A review. Agriculture and Ecological Research Unit, Biological Sciences Division, Indian Statistical Institute, Kolkata, India. 3(7): 905-917. <http://dx.doi.org/10.4236/as.2012.37110>.
- Ahmad, A., Aslam, Z., Iqbal, N., Idrees, M., Bellitürk, K., Rehman, S. U., Ameer, H., Ibrahim, M. U., Samiullah and Rehan, M. (2019). Effect of Exogenous Application of Osmolytes on Growth and Yield of Wheat Under Drought Conditions. *Journal of Environmental and Agricultural Sciences*, 21: 6-13.
- Aira, M., Gómez-Brandón, M., González-Porto P. and Domínguez, J. (2011). Selective Reduction of the Pathogenic Load of Cow Manure in an Industrial-Scale Continuous-Feeding Vermireactor. *Bioresource Technology*, 102: 9633-9637.
- Aira, M., Monroy, F., Dominguez, J. and Mato, S. (2000). How Earthworm Density Affects Microbial Biomass and Activity in Pig Manure. *European Journal of Soil Biology*, 38: 7-10.
- Albanell, E., Plaixats, J. and Cabrero, T. (1988). Chemical Changes During Vermicomposting (*Eisenia fetida*) of Sheep Manure Mixed with Cotton Industrial Wastes. *Biology and Fertility of Soils*, 6: 266-269.
- Arancon, N. Q., Edwards, C. A. and Lee, S. (2002). Management of Plant Parasitic Nematode Populations by Use of Vermicompost. Proc. Brighton Crop Prot. Conf. – Pests and Diseases. 8B-2: 705-716.
- Arancon, N. Q., Edwards, C. A., Bierman, P., Metzger, J., Lee, S. and Welch, C. (2002). Applications of Vermicompost to Tomatoes and Peppers Grown in the Field and Strawberries Grown Under High Plastic Tunnels. Proceedings of the International Earthworm Symposium, Cardiff Wales. September, 2002.
- Arshad, M. and Frankenberger, W. T. (1993). Microbial Production of Plant Growth Regulators. In *Soil Microbial Ecology: Applications in Agricultural and Environmental Management*. Ed. F. B. Metting Jr. Marcell Dekker, New York, Basel, Hong Kong, 307.
- Aruna, G., Kavitha, B., Subashini, N. and Indira, S. (2018). An Observational Study on Practices of Disposal of Waste Garbages in Kamakshi Nagar at Nellore. *International Journal of Applied Research*, 4: 392-394.
- Athani S. I., Hulamanai N. C. and Shirol A. M. 1999. Effect of Vermicompost on the Maturity and Yield of Banana cv. RAJAPURI (Musa AAB)." *South Indian Horticulture*, 47: 4-7.
- Atiyeh R. M., Edwards C. A., Subler S., & Metzger J. D. (2001b). Pig Manure Vermicompost as A Component of a Horticultural Bedding Plant Medium: Effects on Physicochemical Properties and Plant Growth. *Bioresource Technology*, 78: 11-20.
- Atiyeh, R. M., Arancon, N. Q., Edwards, C. A. and Metzger, J. D. (2001a). The Influence of Earthworm-Processed Pig Manure on the Growth and Productivity of Marigolds. *Bioresource Technology*, 81: 103-108.

- Atiyeh, R. M., Dominguez, J., Subler, S. and Edwards, C. A. (2000). Changes in Biochemical Properties of Cow Manure During Processing by Earthworms (*Eisenia andrei*, Bouché) and the Effects on Seedling Growth. *Pedobiologia*, 44: 709-724.
- Ayilara, M. S., Olanrewaju, O.S., Babalola, O. O. & Odeyemi. O. (2020). Waste Management Through Composting: Challenges and Potentials. *Sustainability*, 12(11): 4456.
- Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S. and Smith, D. L. (2018). Plant Growth Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Frontiers in Plant Sciences*, 9: 1473.
- Bansal, S. & Kapoor, K.K. (2000). Vermicomposting of Crop Residues and Cattle Dung with *Eisenia foetida*. *Bioresource Technology*, 73: 95-98.
- Bellitürk, K., 2016. Vermicompost Technology for Solid Waste Management in Sustainable Agricultural Production. *Çukurova Journal of Agriculture and Food Sciences*, 31 (3): 1-5.
- Bellitürk, K., 2018. Some Evaluations about Use of Vermicompost in Agricultural Activity of Thrace Region, Turkey: A Review. *Journal of Rice Research*, 6 (2): 1000193
- Benitez, E., Nogales, R., Elvira, C., Masciandaro, G. and Ceccanti, B. (1999). Enzyme Activities as Indicators of The Stabilization of Sewage Sludge Composting with *Eisenia fetida*. *Bioresource Technology*, 67: 297-303.
- Bhardwaj, D., Ansari, M.W., Sahoo, R.K. and Tuteja, N. (2014). Biofertilizers Function as Key Player in Sustainable Agriculture by Improving Soil Fertility, Plant Tolerance and Crop Productivity. *Microbial Cell Factories*, 13: 1-10.
- Bharti, N., Pandey, S. S., Barnawal, D., Patel, V. K. and Kalra, A. (2016). Plant Growth Promoting Rhizobacteria *Dietzia natronolimnaea* Modulates the Expression of Stress Responsive Genes Providing Protection of Wheat from Salinity Stress. *Scientific Reports*, 6, 34768.
- Borkar, S. G. (2015). *Microbes as Bio-Fertilizers and Their Production Technology*, 1st ed.; WPI Publishing: New York, NY, USA.
- Butt, K. R. (1993). Utilization of Solid Paper Mill Sludge and Spent Brewery Yeast as a Feed for Soil-Dwelling Earthworms. *Bioresource Technology*, 44: 105-107.
- Chanyasak, V., Katayama, A., Hirai, M. F., Mori, S., Kubota, H. (1983). Effects of Compost Maturity on Growth of Komatsuna (*Brassica rapa* Var. *Pervidis*) In Neubauer's Pot: I. Comparison of Growth in Compost Treatments with That in Inorganic Nutrient Treatments as Controls. *Soil Science and Plant Nutrition*, 29(3): 239-250.
- Chaoui, H., Edwards, C. A., Brickner, A., Lee, S. and Arancon, N. Q. (2002). Suppression of the plant parasitic diseases: Pythium (damping off), Rhizoctonia (root rot) and Verticillium (wilt) by vermicompost. Proc. Brighton Crop Prot. Conf. – Pests and Diseases, 8B-3: 711-716.

- Chawngthu, L., Hnamte, R. and Lalfakzuala, R. (2020). Isolation and Characterization of Rhizospheric Phosphate Solubilizing Bacteria from Wetland Paddy Field of Mizoram, India. *Geomicrobiology Journal*, 37: 366-375.
- Chen Y. and Aviad, T. (1990). Effects of humic substances on plant growth. In: MacCarthy, P., C.E. Clapp, R.L. Malcolm and P.R. Bloom (eds) *Humic Substances in Soil and Crop Sciences: Selected Readings*. ASA and SSSA, Madison, Wisconsin, USA, 161-186.
- Coelho, L., Osório, J., Beltrão, J. and Reis, M. (2019). Organic Compost Effects on *Stevia rebaudiana* Weed Control and on Soil Properties in the Mediterranean Region. *Revista de Ciências Agrárias*, 42: 109-121.
- Cook, S. M. F. and Linden, D. R. (1996). Effect of Food Type and Placement on Earthworm (*Aporrectodea tuberculata*) Burrowing and Soil Turnover. *Biology and Fertility of Soils*, 21(3): 201-206.
- Devi, D., Agarwal S. K. and Dayal, D. (1998). Response of Sunflower (*Helianthus annuus* L.) to Organic Manures and Fertilizers. *Indian Journal of Agronomy*, 43(3): 469-473.
- Di Simone, C. D., Sayer, J. A. and Gadd, G. M. (1998). Solubilization of Zinc Phosphate by a Strain of *Pseudomonas fluorescens* Isolated from a Forest Soil. *Biology and Fertility of Soils*, 28(1): 87-94.
- Dineshkumar, R., Kumaravel, R., Gopalsamy, J., Sikder, M. N. A. and Sampathkumar, P. (2018). Microalgae as Bio-Fertilizers for Rice Growth and Seed Yield Productivity. *Waste Biomass Valori*, 9: 793-800.
- Dominguez, J. and Edwards, C. A. (2004). Vermicomposting Organic Wastes: A Review. In: *Soil Zoology for Sustainable Development in the 21st Century* (Shakir, S.H., Mikhail, W.Z.A., Eds).
- Edwards C. A. (1998). *Earthworm Ecology*. CRC Press Boca Raton. 389 pp.
- Edwards C. A. and Burrows I. (1988). The Potential of Earthworm Composts as Plant Growth Media. In *Earthworms in Environmental and Waste Management* Ed. C. A., Neuhauser, SPB Academic Publ. b.v. The Netherlands. 211-220.
- Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd-Allah, E. F. and Hashem, A. (2014). Phytohormones and Beneficial Microbes: Essential Components for Plants to Balance Stress and Fitness. *Frontiers in Microbiology*, 8, 2104.
- Elvira C., Sampedro, L., Benitez, E. and Nogales, R. (1998). Vermicomposting of Sludges from Paper Mill and Dairy Industries with *Eisenia Andrei*: A Pilot-Scale Study. *Bioresource Technology*, 63: 205-211.
- Fernandez-L. O. F., Reyes-Varela, V., Martínez-Suares, C., Salomó n-Hernández, G., Yañez-Meneses, J., Ceballos-Ramírez, J. M., Dendooven, L. (2010). Effect of Different Nitrogen Sources on Plant Characteristics and Yield of Common Bean (*Phaseolus vulgaris* L.) *Bioresource Technology*, 101(1): 396-403.
- Fikre, H., Sandhu, K. S. and Singh, P. (2001). Management of White Rot Pea through Organic Amendment Fungicides. *Plant Disease Research*, 16: 193-197.
- García C., Ceccanti, B., Masciandro, G. and Hernandez, T. (1995). Phosphatase and  $\beta$  Glucosidase Activities in Humic Substances from Animal Wastes. *Bioresource Technology*, 53: 79-87.

- Ilangumaran, G. and Smith, D. L. (2017). Plant Growth Promoting Rhizobacteria in Amelioration of Salinity Stress: A Systems Biology Perspective. *Frontiers in Plant Science*, 8: 1768.
- Itelima, J., Bang, W. J., Onyimba, I. A., Sila, M. D. and Egbere, O. J. (2018). Bio-Fertilizers as Key Player in Enhancing Soil Fertility and Crop Productivity: A Review. *Journal of Microbiology and Biotechnology Reports*, 2: 22-28.
- Jagnow, G. (1987). Inoculation of Cereal Crops and Forage Grasses with Nitrogen-Fixing Rhizosphere Bacteria. Possible Causes of Success and Failure with Regard to Yield Response--A Review. *Z. Pflanzenernähr Bodenkol*, 150: 361-368.
- Joshi. R., Singh, J. & Vig, A. P. (2014). Vermicompost as an Effective Organic Fertilizer and Biocontrol Agent: Effect on Growth, Yield and Quality of Plants. *Reviews in Environmental Science and Biotechnology*. 14(1): 137-159. DOI 10.1007/s11157-014-9347-1.
- Kale, R. D., Mallesh, B. C., Kubra, B. and Bagyaraj, D. J. (1992). Influence of Vermicompost Application on the Available Macronutrients and Selected Microbial Populations in a Paddy Field. *Soil Biology and Biochemistry*, 24: 1317-1320. doi:10.1016/0038-0717(92)90111-A
- Kiehl, J. C. (2001). Produces it from Organic Compound and Vermicompost. *Agricultural Report, Belo Horizonte*, 22 (212): 40-52.
- Kolte U.M., Patil A.S. and Tumberbe A.D. (1999). Response of Tomato Crop to Different Modes of Nutrient Input and Irrigation. *Journal of Maharashtra Agricultural Universities*, 14: 1, 4-8.
- Korir, H., Mungai, N.W., Thuita, M., Hamba, Y. and Masso, C. (2017). Co-Inoculation Effect of Rhizobia and Plant Growth Promoting Rhizobacteria on Common Bean Growth in a Low Phosphorus Soil. *Frontiers in Plant Sciences*, 8: 141.
- Kucey, R. M. N. (1988). Phosphate-Solubilizing Bacteria and Fungi in Various Cultivated and Virgin Alberta Soils. *Canadian Journal of Soil Science*, 63(4): 671-678.
- Kumar, A. S., Meenakumari, K. S. and Anith, K. N. (2017). Screening for Zn Solubilization Potential of Soil Bacteria from Zn Deficient Soils of Kerala. *Journal of Tropical Agriculture*, 54(2): 194.
- Kumar, S. M., Reddy, C. G., Phogat, M. and Korav, S. (2018). Role of Bio-Fertilizers Towards Sustainable Agricultural Development: A Review. *Journal of Pharmacology, Phytochemistry*, 7: 1915-1921.
- Lasaridi, K. E., Manios, T., Stamatiadis, S., Chroni, C. and Kyriacou, A. (2018). The Evaluation of Hazards to Man and the Environment During the Composting of Sewage Sludge. *Sustainability*, 10: 2618.
- Lazarovitis, G., Tenuta, M., Conn, K.L. (2001). Organic Amendments as a Disease Control Strategy for Soilborne Disease of High-Value Agricultural Crops. *Australasian Plant Pathology*, 30: 111-117.
- Mahajan, A. and Gupta, R. D. (2009). Bio-Fertilizers: Their Kinds and Requirement in India. In Integrated Nutrient Management (INM) in a Sustainable Rice-Wheat Cropping System; Mahajan, A., Gupta, R.D., Eds.; Springer: Dordrecht, The Netherlands., 75-100.

- Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A. and Tribedi, P. (2017). Biofertilizers: A potential approach for sustainable agriculture development. *Environmental Science and Pollution Research*, 24: 3315-3335.
- Masciandaro G., Ceccanti, B. and Garcia, C. (1997). Soil Agro-Ecological Management: Fert irrigation and Vermicompost Treatments. *Bioresource Technology*, 59: 199-206.
- Masciandaro, G., Ceccanti, B. and Garcia, C. (1997). Soil Agro-Ecological Management: Fertirrigation and Vermicompost Treatments. *Bioresource Technology*, 59: 199-206.
- Meena, M., Swapnil, P., Divyanshu, K., Kumar, S., Tripathi, Y. K., Zehra, A., Marwal, A. and Upadhyay, R. S. (2020). PGPR-Mediated Induction of Systemic Resistance and Physiochemical Alterations in Plants Against the Pathogens: Current Perspectives. *Journal of Basic Microbiology*, 60: 828-861.
- Mitchell, A. (1997). Production of *Eisenia Fetida* and Vermicompost from Feedlot Cattle Manure. *Soil Biology and Biochemistry*, 29: 763-766.
- Moradi, H., Fahramand, M., Sobhkhizi1, A., Adibian, M., Noori, M., Abdollahi, S. and Rigi, K. (2014). Effect of Vermicompost on Plant Growth and its Relationship with Soil Properties. *International Journal of Farming and Allied Sciences*, 3(3): 333-338.
- Mrinal S., Rajkhowa D. J. & Saikia, M. (1998). Effect of planting density and vermicomposts on yield of potato raised from seedling tubers. *Journal of the Indian Potato Association*. 25, 3-4, 141-142.
- Nair S. K., Naseema, A., Meenakumari, S. K., Prabhakumari, P. and Peethambaran, C. K. (1997). Microflora Associated with Earthworms and Vermicomposting. *Journal of Tropical Agriculture*, 35: 93-98.
- Nakasone, A. K., Bettiol, W. and de Souza, R. M. (1999). The Effect of Water Extracts of Organic Matter on Plant Pathogens. *Summa Phytopathologica*, 25(4): 330-335.
- Nielson R. L. (1965). Presence of Plant Growth Substances in Earthworms Demonstrated by Paper Chromatography and the Went Pea Test. *Nature Lond*, 208: 1113-1114.
- Norman, Q., Arancon, Clive, A. and Edwards. (2005). Effects of Vermicomposts on Plant Growth. Paper Presented During the International Symposium Workshop on Vermi Technologies for Developing Countries (ISMVT 2205), November 16-18. Los Banos, Philippines.
- Orozco, S. H., Cegarra, J., Trujillo, L. M. and Roig, A. (1996). Vermicomposting of Coffee Pulp Using the Earthworm *Eiseniafetida*: Effects on C and N Contents and the Availability of Nutrients. *Biology and Fertility of Soils*, 22: 162-166.
- Palanisamy, S. (1996). Earthworm and Plant Interactions. ICAR Training Program, Tamil Nadu Agricultural University, Coimbatore.
- Pathma, J. and Natarajan, S. (2012). Microbial Diversity of Vermicompost Bacteria that Exhibit Useful Agricultural Traits and Waste Management Potential. Springer Plus, 1: 1-19.
- Patil, M. P., Humani, N. C., Athani, S. I. and Patil, M. G. (1998). Response of New Tomato Genotype Megha to Integrated Nutrient Management. *Advances in*

- Agricultural Research in India* 9: 39-42.
- Raklami, A., Bechtaoui, N., Tahiri, A., Anli, M., Meddich, A. and Oufdou, K. (2019). Use of Rhizobacteria and Mycorrhizae Consortium in the Open Field as a Strategy for Improving Crop Nutrition, Productivity and Soil Fertility. *Frontiers in Microbiology*, 10: 1106.
- Ramachandra, R., Reddy M. A. N., Reddy Y. T. N., Reddy N. S., Anjanappa M. and Reddy, R. (1998). Effect of Organic and Inorganic Sources of NPK on Growth and Yield of Pea (*Pisum sativum*). *Legume Research*, 21(1): 57-60.
- Rani, N., Kaur, R. and Kaur, S. (2020). Zinc Solubilizing Bacteria to Augment Soil Fertility—A Comprehensive Review. *International Journal of Agricultural and Veterinary Sciences*, 8(1): 38-44.
- Rawat, P., Das, S., Shankhdhar, D. and Shankhdhar, S. C. (2020). Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. *Journal of Soil Science and Plant Nutrition*, 1-20.
- Reeh, U. (1992). Influence of Population Densities on Growth and Reproduction of the Earthworm *Eisenia andrei* on Pig Manure. *Soil Biology and Biochemistry*, 24: 1327-1331.
- Reinecke, A., Viljoen, S. V. and Saayman, R. (1992). The Suitability of *Eudrilus eugenie*, *Perionyx excavatus* and *Eisenia Fetida* (Oligochaeta) for Vermicomposting in Southern Africa in Terms of Their Temperature Requirements. *Soil Biology and Biochemistry*, 24: 1295-1307. doi:10.1016/0038-0717(92)90109-B.
- Ritika, B. and Utpal, D. (2014). Biofertilizer, a Way Towards Organic Agriculture: A Review. *African Journal of Microbiology Research*, 8: 2332-2343.
- Rodriguez, J. A., Zavaleta, E., Sanchez, P. and Gonzalez, H. (2000). The effect of vermicomposts on plant nutrition, yield and incidence of root and crown rot of gerbera (*Gerbera jamesonii* H. Bolus). *Fitopatol*, 35: 66-79.
- Ruz-Jerez, B. E., Ball, P. R. and Tillman, R. W. (1992). Laboratory Assessment of Nutrient Release from a Pasture Soil Receiving Grass Or Clover Residues, in the Presence or Absence of *Lumbricus rubellus* or *Eisenia fetida*. *Soil Biology and Biochemistry*, 24: 1529-1534. doi:10.1016/0038-0717(92)90145-N.
- Sallaku, G., Babaj, I., Kaciu, S. and Balliu, A. (2009). The Influence of Vermicompost on Plant Growth Characteristics of Cucumber (*Cucumis sativus* L.) Seedlings under Saline Conditions. *Journal of Food, Agriculture and Environment*, 7: 869-872.
- Saravanan, V. S., Subramoniam, S. R. and Raj, S. A. (2004). Assessing in Vitro Solubilization Potential of Different Zinc Solubilizing Bacterial (ZSB) Isolates. *Brazilian Journal of Microbiology*, 35(1-2): 121-125.
- Schütz, L., Gattinger, A., Meier, M., Müller, A., Boller, T., Mäder, P. and Mathimaran, N. (2018). Improving Crop Yield and Nutrient Use Efficiency Via Biofertilization—A Global Meta-Analysis. *Frontiers in Plant Sciences*, 8: 2204.
- Senesi N., Saiz-Jimenez, C. and Miano, T. M. (1992). Spectroscopic Characterization of Metal-Humic Acid-Like Complexes of Earthworm-Composted Organic Wastes. *The Science of the Total Environment*, 117/118: 111- 120.
- Shakir S. H. and Mikhal, W. Z. A. (2004). The Influence of Vermicompost on Plant

- Growth and Pest Incidence Soil Zoology for Sustainable Development in the 21th Century. Cairo.
- Sharma, K. and Garg, V.K. (2017). Management of Food and Vegetable Processing Waste Spiked with Buffalo Waste Using Earthworms (*Eisenia fetida*). *Environmental Science and Pollution Research*, 24(8): 7829-7836. 10.1007/s11356-017-8438-2.
- Sharma, S., Kulkarni, J. and Jha, B. (2016). Halotolerant Rhizobacteria Promote Growth and Enhance Salinity Tolerance in Peanut. *Frontiers in Microbiology*, 7: 1600.
- Singh, C. M., Sharma, P. K., Kishor, P., Mishra, P. K., Singh, A. P., Verma, R. and Raha, P. (2011). Impact of Integrated Nutrient Management on Growth, Yield and Nutrient Uptake by Wheat (*Triticum aestivum* L). *Asian Journal of Agricultural Research*, 7: 1-7.
- Sreenivas, C., Muralidhar S. and Rao M. S. (2000). Vermicompost: A Viable Component of IPNSS in Nitrogen Nutrition of Ridge Gourd. *Annals of Agricultural Research*. 21: 108-113.
- Sun, B., Bai, Z., Bao, L., Xue, L., Zhang, S., Wei, Y., Zhang, Z., Zhuang, G. and Zhuang, X. (2020). *Bacillus subtilis* Biofertilizer Mitigating Agricultural Ammonia Emission and Shifting Soil Nitrogen Cycling Microbiomes. *Environment International*, 144: 105989.
- Suthar, S. (2010) Vermicompost: An Environmentally Safe, Economically Viable and Socially Acceptable Nutritive Fertilizer for Sustainable Farming; In: Sinha, R.K., et al., Eds., Special Issue on Vermiculture Technology, *Journal of Environmental Engineering, Inderscience Publishing, Olney*.
- Szczecz, M. (2002). Induction of Systemic Resistance in Radish by Pseudomonads. Developing Iri Vermicomposts Amended Substrate. *Phytopathologia Polonica*, 24: 57-66.
- Timmusk, S., Abd El-Daim, I. A., Copolovici, L., Tanilas, T., Kännaste, A., Behers, L., Nevo, E., Seisenbaeva, G., Stenström, E. and Niinemets, U. (2014). Drought-Tolerance of Wheat Improved by Rhizosphere Bacteria from Harsh Environments: Enhanced Biomass Production and Reduced Emissions of Stress Volatiles. *PLoS ONE*, 9: e96086.
- Timmusk, S., Kim, S. B., Nevo, E., Abd El Daim, I., Ek, B., Bergquist, J. and Behers, L. (2015). Sfp-Type Pptase Inactivation Promotes Bacterial Biofilm Formation and Ability to Enhance Wheat Drought Tolerance. *Frontiers in Microbiology*, 6: 387.
- Tomati, U., Grappelli, A. and Galli, E. (1983). Fertility Factors in Earthworm Humus. Proc. Int. Symp. Agric. Environ. Prospects in Earthworm Farming. Publication Ministero della Ricerca Scientifica e Tecnologia, Rome, 49-56.
- Trivedi, P. & Sa, T. (2008). *Pseudomonas corrugata* (NRRL B-30409) Mutants Increased Phosphate Solubilization, Organic Acid Production and Plant Growth at Lower Temperatures. *Current Microbiology*, 56: 140-144.
- Tyler, H. H., Warren, S. L., Bilderback, T. E., and Fonteno, W. C. (1993). Composted Turkey Litter: I. Effect on Chemical and Physical Properties of a Pine Bark Substrate. *Journal of Environmental Horticulture*, 11(3): 131-136.
- Ushakumari, K., Prabhakumari, P. and Padmaja P. (1999). Efficiency of

- Vermicomposts on Growth and Yield of Summer Crop Okra (*Abelmoschus esculentus* Moench). *Journal of Tropical Agriculture*, 37: 87-88.
- Uyizeye, O. C., Rachel K., Thiet, Melissa, A. and Knorr. (2019). Effects of Community-Accessible Biochar and Compost on Diesel-Contaminated Soil. *Bioremediation Journal*, 23(2): 107-117. DOI: 10.1080/10889868.2019.1603139.
- Venkatesh, Patil P. B., Patil C. V. and Giraddi R. S. (1998). Effect of In-Situ Vermiculture and Vermicomposts on Availability and Plant Concentration of Major Nutrients in Grapes. *Karnataka Journal of Agricultural Sciences*, 11: 117-121.
- Ventorino, V., Pascale, A., Fagnano, M., Adamo, P., Faraco, V., Rocco, C., Fiorentino, N. and Pepe, O. (2019). Soil Tillage and Compost Amendment Promote Bioremediation and Biofertility of Polluted Area. *Journal of Cleaner Production*, 239: 118087.
- Vessey, J. K. (2003). Plant Growth Promoting Rhizobacteria as Biofertilizers. *Plant Soil*, 255: 571-586.
- Vyas, P. and Gulati, A. (2009). Organic Acid Production In-Vitro and Plant Growth Promotion in Maize Under Controlled Environment by Phosphate-Solubilizing Fluorescent *Pseudomonas*. *BMC Microbiology*, 9: 1-15.
- Werner, M. and Cuevas, R. (1996). Vermiculture in Cuba. *Biocycle*. Emmaus, PA., JG Press. 37: 61-62.
- Wiegant, W. M. A. (1992). Simple Method to Estimate the Biomass of Thermophilic Fungi in Composts. *Biotechnology Techniques*, 5(6): 421-426.
- Zandi, P. and Basu, S. K. (2016). Role of Plant Growth-Promoting Rhizobacteria (PGPR) as Biofertilizers in Stabilizing Agricultural Ecosystems. In *Organic Farming for Sustainable Agriculture*; Nandwani, D., Ed.; Springer: Cham, Switzerland, 71-87.
- Zende, G. K., Ruikar, S. K. and Joshi, S. N. (1998). Effect of Application of Vermicomposts Along with Chemical Fertilizers on Sugar Cane Yield and Juice Quality. *Indian Sugar*, 48: 357-369.

## CHAPTER 2

### AN OVERVIEW OF HAPLOID PLANT PRODUCTION IN CUCURBITS

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## INTRODUCTION

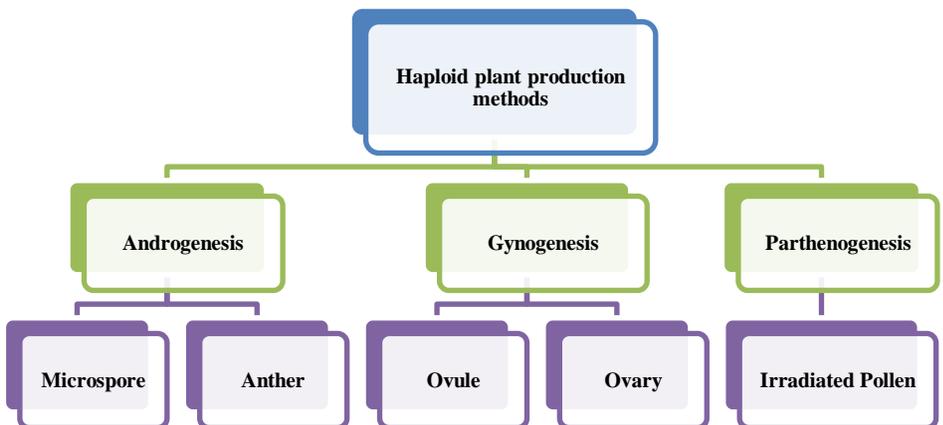
*Cucurbitaceae* is one of the most potential plant families, which has approximately 975 species within around 98 genera, which include several economically important and popular fruit and vegetables, frequently disseminated in tropical and sub-tropical areas worldwide (Xu and Chang, 2017; Salehi et al. 2021). The most nutritional and potential horticultural crops in this family are melon (*Cucumis melo* L.), pumpkin (*Cucurbita moschata* Duchesne ex Poir.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), bottle gourd (*Lagenaria siceraria*), cucumber (*Cucumis sativus* var. *sativus* L.), summer squash (*Cucurbita pepo* L.) and winter squash (*Cucurbita maxima* Duch. ex. Lam.) species (Mandoulakani et al. 2015; Dhillon et al. 2016). Breeding is focused on modifying the genetic structure of presenting varieties to create desirable characteristics such as phenotypes and genotypes for a given objective. Breeding with new technologies has been applied to increase the production and develop the quality of primary vegetables along with to meet customer's preference and demands and providing appropriate cultivars to the farmer in short of time. Haploid and double haploid production as part of advanced breeding approved to be popular among breeders in the latest years and also utilized in cucurbits breeding studies to increase the 100% pure homozygous lines required (Nyirahabimana and Solmaz, 2022). The inbred pure lines production is known as the fundamental of modern vegetable breeding. Breeding programs take a very long time due to the classical breeding of the vegetable species belonging to the *Cucurbitaceae* family (Zheng et al. 2019).

For reasons such as shortening this period, facilitating selection, and increasing breeding efficiency, the haploid plant production methods have been used in cucurbit crops. Biotechnological methods are also used in the breeding studies of cucumber, melon, winter squash and summer squash,

watermelon, bottle gourd, pumpkin, which are the most important vegetables (Dhillon et al. 2020). Generally, the haploid plant is the plant that contains with a gametophyte chromosome number ( $n$ ) and doubled haploids ( $2n$ ) are haploid plants which have been subjected to chromosome duplication. However, seeds are used to propagate cucurbits, and the traditional breeding methods have been used to increase these vegetable crops are difficult and have various limitations (Sultana et al. 2021). Furthermore, haploids and DHs production, as well as the evolution of haploidy technology to provide homozygous plants, is a very appealing biotechnological technique. Recently, the available methods to obtain haploids plants in cucurbits are *in vitro* androgenesis, gynogenesis that are added to the pollination with irradiated pollens i.e parthenogenesis and are most effective and widely applied techniques (Dong et al. 2016; Sari and Solmaz, 2021).

## 1. HAPLOID PLANT PRODUCTION TECHNIQUES IN CUCURBIT CROPS

This section enumerates all techniques used in *Cucurbitaceae* crops improvement for advanced breeding as well as androgenesis, gynogenesis, and parthenogenesis techniques.



**Figure 1:** Haploid Plant Production Techniques in *Cucurbitaceae* Crops

### **1.1. Anther-microspore Culture (Androgenesis) Technique**

According to androgenesis (in vitro anther/microspore culture) method, several factors are considered to have distinct effects on haploid production through this process such as genotype, environmental factor, culture conditions such as pre-treatments applications (cold and heat shocks), plant growth regulators or hormones (Nyirahabimana and Solmaz, 2021). Commonly, it is known that many factors influence are donor plant genotypes, heat shock and cold pre-treatments of male flower buds, during *in vitro* androgenesis culture processes, development stages of anther/microspore in culture, the growing conditions of donor plant, embryo developmental induction, regeneration, and differentiation media, and chromosome doubling strategies (Sari and Solmaz, 2021). The *in vitro* androgenesis such as anther culture is generally an alternative technique for haploid and DHs production in several crops owning that it may be established and applied on the large-scale for a wide range of genotypes (Seguí-Simarro and Nuez, 2004). For instance, the effect of hormones (2,4-D) and sucrose combinations at different concentrations on the formation of haploid plants in summer squash variety via anther culture has been analysed. The used basal medium was MS (Murashige and Skoog) and different concentrations of sugar 30, 60, 90, 120 and 150 g L<sup>-1</sup> and 2,4-D (0.1, 1.0, 2.5 and 5.0 mg L<sup>-1</sup>) were compared. The obtained results have been reported that mostly the plantlets were formed from the incubation medium supported by 2,4-D (5 mg L<sup>-1</sup>) and sucrose (150 gL<sup>-1</sup>) ( Gałazka and Niemirowicz-Szczytt, 2013). Nowadays, most studies recommend that anther culture is similar in efficacy for pollination with irradiated pollen method. For instance: several research based on anther/microspore culture in cucumber have been performed to produce haploid (Kumar et al. 2003; Mohamed and Refaei, 2004; Song et al. 2007; Suprunova and Shmykova,

2008; El-Maksoud et al. 2009; Zhan et al. 2009; Nguyen and Chen, 2012; Hamidvand et al. 2013; Gałazka and Niemirowicz-Szczytt, 2013; Abdollahi et al. 2016; Asadi et al. 2018; Asadi et al. 2019; Amirian et al. 2020; Ghanbari and Golabadi, 2020; Asadi and Seguí-Simarro, 2021), watermelon ( Yue et al. 2005; Zhu et al. 2010; Zhu et al. 2012; Abdollahi et al. 2015; Zhu et al. 2015; Akbaş and Solmaz, 2019; da Silva et al. 2021; Sari and Solmaz, 2021), melon (Dong et al. 2016; Nguyen et al. 2019; de Oliveira et al. 2022); winter squash and pumpkin (Kurtar et al. 2016; Kurtar, 2018; Kurtar et al. 2020; Kurtar and Seymen, 2021c). **Table 1** highlights recent and current studies to generate haploid and DH plants in *Cucurbitaceae* family through androgenesis techniques.

**Table 1:** Recent Summary of Haploid Plant Induction via Androgenesis in Cucurbits

Species	Method	Cold pre-treatment (Day=d)	Heat shock (Day=d, weeks=w)	Induction medium (PGR)	Regeneration medium (PGR)	Results (plantlets)	Reference
Squash (summer & winter)	AC	4°C / 2 d	35°C / 2 d	MS + NAA + BA	MS + BA	HP, DH	Kurtar et al. 2016
<i>Cucurbita</i> species	AC	4°C / 2 d	35°C / 7 d	MS + 2,4-D	MS + KIN + NAA	HP, DH	Rakha et al. 2012; Kurtar and Seymen, 2021c
Cucumber	AC	4°C (un-specified)	33°C / 1 hour	MS + BA + 2,4-D + KIN	MS + BA	HP, DH	Song et al. 2007
	AC		35°C / 72 hours	MS + 2,4-D + BA	MS + BA + NAA	HP, DH	Suprunova and Shnykova, 2008
	MC		22°C (in dark)	NLN + 2,4-D		HP	
	MC	4°C / 2 d	33°C / 1 d	NLN + 2,4-D + BA	MS + BA	HP, DH	Zhan et al. 2009
	AC		25°C / 4 d	MS + NAA + BA + 2,4-D	MS + BA + NAA	HP, DH	Hamidvand et al. 2013
	AC		25°C / 30-35 d	2,4-D + BA + KIN	MS + NAA + BA	DH, HP	Abdollahi et al. 2016
	AC	4°C / 2 d	35°C / 1 hour then; 25°C / 20 d	BAP + 2,4-D	2,4-D + BA	DH, HP	Asadi et al. 2018
Watermelon	AC	4°C / 2 d	35°C / 2 d	MS + NAA + BA	MS + BA	HP, DH	Abdollahi et al. 2015
Melon ( <i>Momordica charantia</i> L.)			25°C / 4 w	1.0 mgL <sup>-1</sup> 2,4-D and 1.5 mgL <sup>-1</sup> BA; 1.5 mgL <sup>-1</sup> 2,4-D and 1.0 mgL <sup>-1</sup> BA; 1.5 mgL <sup>-1</sup> NAA, 1.0 mgL <sup>-1</sup> BA and 0.5 mgL <sup>-1</sup> KIN			Nguyen et al. 2019

**AC:** Anther culture, **DH:** Double Haploid, **HP:** Haploid plantlet, **MC:** Microspore culture

## 1.2. Un-pollinated Ovule and Ovary Culture (Gynogenesis) Technique

Recently, in vitro gynogenesis become a prominent technique applied to produce haploid/double-haploid or suitable homozygous lines for advanced vegetable breeding programs (Kurtar and Seymen, 2021a). According to its facility, gynogenesis is used as an alternative haploid induction method compared to the application of irradiated pollen technique which requires an external beam source and exists with intense labor during embryo recovery (Zhu et al. 2020). Additionally, the ovule-ovary culture in haploid production studies for distinct cucurbits genotypes reported to be affected by several factors including genotype, temperature shock (cold/heat) applications, female gametophyte developmental stages, plant growth regulators (Gałaszka and Niemirowicz-Szczytt, 2013; Golabadi et al. 2017; Ozsan et al. 2017; Nyirahabimana and Solmaz, 2021; Baktemur et al. 2022).

Currently, studies of haploid plant production through gynogenesis in cucurbits have intensified, for instance: watermelon (Zhu et al. 2018; Zou et al. 2018; Zhu et al. 2020); pumpkin and squash ( Min et al. 2016; Zou et al. 2020); cucumber (Li et al. 2013; Moqbeli et al. 2013; Tantasawat et al. 2015; Golabadi et al. 2017; Ozsan et al. 2017; Erol and Sari, 2019; Deng et al. 2020; Demirel and Onus, 2021; Baktemur et al. 2022); melon (Nitwatthanakul and Tiraumphon, 2018). Currently, the evaluation of double haploid production in *Cucurbitaceae* crops have taken in action (Domblides et al. 2019; Kurtar and Seymen 2021a; Sari and Solmaz, 2021; Segui-Simarro, 2021). **Table 2** highlights recent and current studies to produce haploid and DH plants in *Cucurbitaceae* through the gynogenesis method.

Table 2: Recent Summary of Haploid Plant Induction via Gynogenesis in Cucurbits

Species	Explant	Development stage (days=d/ hours=h)	Cold treatment (days=d)	Pre-treatment	Heat shock (days=d, weeks=w)	Induction (PGRs)	medium	Regeneration medium (PGRs)	Result (plantlets)	Reference
Squash (summer winter)	Ovules	1 day prior to anthesis	4°C, 0 d		25°C, 4 w	MS + 2,4-D +NAA + BAP		N6	CP, HP, DH	Xie et al. 2006
	Ovules	1 day before anthesis	4°C, 4 d		32°C, 4 d	MS + KIN + 2,4-D		MS	HP, DH	Shalaby, 2007
	Ovary	Day of anthesis	4°C, (d/w unspecified)	un-specified	35°C, 5 d		MS + Sugar + Agar	MS + Sugar + Agar	HP, CP, DP, TP	Zou et al. 2020
Pumpkin	Ovules	1 day before anthesis			35°C, 6 d	MS + BAP + 2,4-D + NAA		MS + BAP + NAA		Sun et al. 2009
	Ovules	Day of anthesis			35°C, 5 d	MS + TDZ		MS	HP, DH	Min et al. 2016
Other cucurbits	Ovules	1 day before anthesis	4°C, 0 d		25°C, 4 w	MS + 2,4-D		MS	HP, DH	Rakha et al. 2012
	Ovary	Day of anthesis	4°C, (d/w unspecified)	un-specified	35°C, 5 d			MS + Sugar + Agar	HP, CP, DP, TP	Zou et al. 2020
	Ovary	1 day before anthesis			35°C, 5 d	TDZ, NAA, 6-BA, KT, and casein hydrolysate			HP, DH, TP	Zhu et al. 2019
	Ovules	1 day before anthesis 2 days before anthesis			35°C, 3 d	MS + TDZ + 2,4-D + SPD/PUT		MS + TDZ + 2,4-D	HP	Yildiz and Solmaz, 2020
Watermelon	Ovary	Day of anthesis	4°C, 4 d		25°C, 4 w	MS + TDZ		MS + BAP	HP, DH	Malik et al. 2011
Melon	Ovaries	1 day before anthesis			25°C, 3 d	MS + BAP + NAA		MS + BAP	HP, DH	Koli and Murthy, 2013
	Ovaries	6h before anthesis	5°C, 2 d		35°C, 2-4 d	CBM + TDZ		CBM + NAA + BAP	CP, HP, DH	Gemesne-Juhasz et al. 2002
Cucumber	Ovules	6h before anthesis			22°C (d/w unspecified)	MS + TDZ + BAP		MS + NAA + BAP	HP, DH	Suprunova and Shmykova, 2008
	Ovaries	1 day before anthesis	5°C, 2 d		35°C, 3 d	MS + TDZ		MS + BAP	HP, DH, TP	Diao et al. 2009
	Ovaries	At anthesis day			35°C, 4 d	CBM + TDZ		CBM + NAA + BAP	CP, HP, DH	Li et al. 2013
	Ovaries	1 day before anthesis 1 day before anthesis	5°C, 2 d		35°C, 3 d 25°C (d/w unspecified)	MS + TDZ CBM + AgNO3		MS + NAA + BAP MS + BAP + IAA	HP, DH HP, DH	Mogbeli et al. 2013 Piapung et al. 2014a, b
	Ovaries	1 day before anthesis			35°C, 3 d	MS + TDZ + BAP		MS + BAP + NAA	HP	Tanatsawa et al. 2015
	Ovaries	1 day before anthesis			35°C, 3 d	CBM + NAA + BAP		CBM + TDZ	HP	Ozsan et al. 2017
	Ovaries	1 day before anthesis			35±1°C, 3 d	TDZ (0.01-0.08)		(GA3 + NAA) and BAP		Asadi et al. 2019
	Ovules	1 day before anthesis			32°C, 7-10 d	MS + TDZ 2,4-D				Dombildes et al. 2019
	Ovules / Ovaries	1 day before anthesis			35°C, 3 d and 25°C, 3 w	MS + TDZ + SPD + PUT		TDZ + BAP	HP	Erol and Sari, 2019
	Ovaries	1 day before anthesis	4°C, 5 d		TDZ			TDZ	HP, DH	Deng et al. 2020
	Ovaries	1 day before anthesis			TDZ, and 2,4-D + KIN			NAA + BAP	HP, DH	Baktemur et al. 2022

CP: Chimeric plantlets, DP: Diploid, DH: Double Haploid, HP: Haploid plantlets, TP: tetraploid plantlet

### 1.3. Parthenogenesis Technique

Parthenogenesis or the application of irradiated pollen is the haploid induction that is very popular in haploid production in *Cucurbitaceae* family. After the discovery of first summer melon haploid plant production via parthenogenesis technique by Sauton (1987), the first winter melon (Sari et al. 1992) and watermelon haploids (Sari et al. 1994) were developed by parthenogenesis technique. Authors have noticed that the dose and the age of pollen decreased the pollen viability (Dong et al. 2016). Currently, various studies have been performed for haploid plant production via parthenogenesis method. For instance, Kurtal et al. (2021) applied gamma rays with cobalt<sup>60</sup> source [ $\gamma$  (Co<sup>60</sup>)] and by using medium of E20A (modified) for induction and MS + 1 mg L<sup>-1</sup> IAA + 0.1 mg L<sup>-1</sup> BA for embryo regeneration, the haploid plants have been produced from squashes. Moreover, Lotfi et al. (2008), applied irradiated pollen with 250 Gy dose to the cucumber male flowers. The pollinated fruits have been harvested after 21-23 days from the day of pollination, and the seeds taken from the harvested fruits were transferred to the liquid culture of E20A medium and kept under 16/8 hours light/dark conditions for ten days. Furthermore, Ebrahimzadeh et al. (2021) carried out a research on production of haploid plants in Styrian pumpkin using irradiated pollen by applying gamma [ $\gamma$  (Co<sup>60</sup>)] rays with 100 Gy dose. The rescued embryos have been cultured in an induction medium of E20A + 0.01 mg L<sup>-1</sup> IAA + 2% sucrose which was the same as in embryo regeneration medium.

Additionally, Dal et al. (2016) carried out a study about the application and comparison of effectiveness of various sources of irradiation to stimulate haploid embryos of Kırkağaç melons. Two distinct doses of cesium-137 (200 Gy and 300 Gy), one dose (300 Gy) from cobalt-60, and two various doses (200 Gy and 300 Gy) from X-ray have been applied to

irradiate pollen. The high value (60.38%) of the fruit set has reported from cesium-137 source, with a dose of 200 Gy and the lowest value (20.01%) was observed in the 300 Gy dose X-ray source. In the study performed by Hooghvorst et al. (2020) the use of X-ray ( $^{137}\text{Cs}$ ) radiation with 250 Gy dose to produce haploid and double haploid plants in melon. During embryo rescue the E20A (liquid) was used as an induction medium and E20A (solid) for embryo regeneration medium. Currently, double haploid plant generation in (Piel de Sapo) melon variety via irradiated pollen study has been carried out. The beneficial protocol for producing haploid embryo through parthenogenesis and protocol of haploid plants chromosome doubling for 'Piel de Sapo' genotypes have been developed (Hooghvorst et al. 2021). **Table 3** highlights recent and current studies to produce haploid and DH plants in *Cucurbitaceae* family through pollination with irradiated pollen technique (parthenogenesis).

**Table 3:** Haploid Production Via Pollination with Irradiated Pollen Technique in Cucurbits

Species	Radiation sources	Radiation doses	Development stages of fruit (Day=d, weeks=w)	Embryo excise	Induction media (mgL <sup>-1</sup> )	Regeneration media (mgL <sup>-1</sup> )	Results (plantlets)	Reference
Cucumber	$\gamma$ (Co <sup>60</sup> )	100 - 400 Gy	2 - 35 w	OI	MS	MS + BAP	CP, HP, DH	Lei et al. 2006
	$\gamma$ (Co <sup>60</sup> )	300 Gy	3 - 5 w	OI	E20A	MS	HP, DH	Smiech et al. 2008
	$\gamma$ (Co <sup>60</sup> )	250 Gy	21 - 23 d	LB	E20A			Lotfi and Salehi, 2008
	$\gamma$ (Co <sup>60</sup> )		10 d		E20A		HP, DH	Lotfi et al. 2008
Watermelon	$\gamma$ (Co <sup>60</sup> )	200, 300 Gy	2-4 w	OI	E20A + 0.107 IAA	E20A + 0.107 IAA	HP, DH, TP	Sari et al. 1994
	$\gamma$ (Co <sup>60</sup> )	300 Gy, 150, 200, 250 and 300 Gy	3 w	OI	E20A MS	MS	None	Solmaz et al. 2011a
	$\gamma$ (Co <sup>60</sup> )	275 Gy	25 d	OI	CP (Chee et al.1992) + 0.02 IAA + 3% Sucrose		HP	Taskin et al. 2013
Melon	$\gamma$ (Co <sup>60</sup> )	300 Gy	3 - 4 w	OI, X ray	E20A	E20A	HP, DH	Sari et al. 1992
	$\gamma$ (Co <sup>60</sup> )	300 Gy	21-25 d			E20A	HP, DH, TP	Koksal et al. 2001
	X-rays	65 KR	21 d		MS	MS	HP, DH	Yashiro et al. 2001
	X-rays	130 kR or 65 kR	3 w		MS	MS	HP	Kuzuya et al. 2003
	Cs <sup>137</sup>	250 Gy	21-23 d	OI	E20A	E20A	CP, HP, DH	Lotfi et al. 2003
	Cs <sup>137</sup>	250 Gy	3 w	LMC	E20A	E20A	CP, HP, DH	Lim and Earle, 2008; 2009
	$\gamma$ (Co <sup>60</sup> )	300 Gy	3-4 w	LB	E20A + 0.01 IAA	E20A + 0.5 BAP + 0.01 IAA	CP, HP, DH	Ari et al. 2010
	$\gamma$ (Co <sup>60</sup> )		3-5 w	MI	E20H8	E20H8	HP, DH	Gonzalo et al. 2011
	$\gamma$ (Co <sup>60</sup> )	300 Gy	1 day before anthesis		E20A	E20A	HP, DH	Solmaz et al. 2011
	Cs <sup>137</sup>	250 Gy	3 w	LMC	E20A	E20A	HP, DH	Nasertorabi et al. 2012
	$\gamma$ (Co <sup>60</sup> )	250 Gy	21 d	OI	E20A + 0.011 IAA	E20A + 0.011 IAA	HP, DH	Godbole and Murthy, 2012a, b
	$\gamma$ (Co <sup>60</sup> )	300 Gy	3-4 w	OI	MS		HP	Dal et al. 2016
	Cs <sup>137</sup>	200 Gy and 300 Gy	3-4 w	OI	MS		HP	
	X-ray	200 Gy and 300 Gy	3-4 w	OI	MS		HP	
X-ray (Cs <sup>137</sup> )	250 Gy	3 w		E20A (Liquid)	E20A (Solid)	HP, DH, CP	Hooghvorst et al., 2020	

	$\gamma$ (Co <sup>60</sup> )	300 Gy	3-4 w	OI	E20A	MS	HP, DH	Ivanova, 2020
Squash (summer & winter)	$\gamma$ (Co <sup>60</sup> )	50, 100 Gy	3-4 w	LMC	E20A	E20A	HP, DH	Kurtar and Balkaya, 2010
	$\gamma$ (Co <sup>60</sup> )	150 Gy	35 d	OI	CP medium (Chec et al.1992): (30 g L <sup>-1</sup> sucrose, 8 g L <sup>-1</sup> agar, 0.08 B12 + 0.02 IAA)	E20A	HP, DH	Baktemur et al. 2014
	$\gamma$ (Co <sup>60</sup> )		3-4 w	OI	E20A (modified )	MS + 0.1 IAA + 1 BAP	HP	Kurtar et al. 2021b
Pumpkin and Pumpkin cv. <i>Styriaca</i>	$\gamma$ (Co <sup>60</sup> )	50, 100 Gy	3-6 w	OI	E20A	E20A	HP, DH	Kurtar et al. 2009
	X rays	200 Gy	4 w	OI	E20A + 0.011 IAA	E20A + 0.011 IAA	HP, DH, TP	Kos'mrlj et al. 2013
	$\gamma$ (Co <sup>60</sup> )	50, 75 Gy	4 w	LB	E20A + 0.011 IAA		HP, DH	Ebrahimzade h et al. 2013
	$\gamma$ (Co <sup>60</sup> )	100 Gy	4 w	LB	E20A + 0.01 IAA + 2% Sucrose	E20A + 0.01 IAA + 2% Sucrose	HP, DH	Ebrahimzade h et al. 2021

**CP:** Chimeric (mixoploid) plantlet, **DH:** Double Haploid plantlet,  $\gamma$  (Co<sup>60</sup>): Gamma ray source (Cobalt 60), **HP:** Haploid plantlet, **LB:** light box containing a small white fluorescent lamp, **LMC:** liquid medium culture, **MI:** morphology identification, **OI:** opened individually, **TP:** tetraploid plantlet, **XR:** X-ray radiography

## **2. CHROMOSOME DOUBLING AND PLOIDY LEVEL DETERMINATION**

### **2.1. Ploidy Level Determination**

Generally the most known and useful methods for determining ploidy level are direct method (chromosome counting) and indirect methods (morphological observation, stomatal sizes, chloroplast counts and flow cytometry).

#### **a) Chromosome counts**

Counting for chromosomes in metaphase stage in actively growing cells is the oldest method. Although it can be done in cells at the stage of mitosis or meiosis; counting mitotic chromosomes is faster and easier. Root ends are the very important source of mitotic cells. If root-tips are not suitable, leaves, young buds, or calli cells may also be used. In this method, which requires a long time and effort; the cytological procedures for the preparation and staining of chromosomes vary according to the plant species and if plants are in an *in vivo* or *in vitro* conditions (Song et al. 2007; Mohamed and Refaei, 2004; Abdollahi et al. 2016; Dong et al. 2016). The Sang (2002) technique has been applied for chromosome counts. Nevertheless, chromosomes of several species in this family are too small and hard to determine even if by using a microscope of high power (Zhang et al. 2019a).

#### **b) Morphological Observation and Presence of Pollen**

Haploid plants have the characteristic of being a miniature of the same plant, showing the characteristics of its kind, but with smaller leaves, thinner stems, and shorter stature (Sari et al. 1999). They are also sterile due to their inability to form pollen. It is an old but still valid method to identify the plant by looking at its morphological and physiological characteristics and to

examine the pollen presence daily in greenhouse conditions, especially after the haploid plants are acclimated to the external environment (Kurtar et al. 2002; Xie et al. 2006; Song et al. 2007)

### **c) Stomatal Examinations and Chloroplast Counts**

Stomata are living structures that are formed by the differentiation of the epidermis in plants and that control transpiration and gas exchange in the plant with their opening and closing properties. Stomata consist of two guard cells, and inside the guard cells are chloroplasts, a cytoplasmic organelle called chloroplasts where photosynthesis takes place. It has been proven in many plant species that there is a linear relationship between the sizes of stomata in plants and the chloroplast numbers in guard cells and ploidy levels. Normally, this technique is known as cytological analysis in various studies (Sari et al. 1999; Kurtar and Balkaya, 2010; Min et al. 2016).

### **d) Flow cytometry**

Mostly, in several current studies the ploidy levels of plantlet which regenerated from various plant crops including vegetables is determined by flow cytometry with taking sample fresh or young leaves for several cucurbits (Sari et al. 1999; Claveria et al. 2005; Gonzalo et al. 2011; Li et al. 2013; Zhang et al. 2019b; Zou et al. 2020).

## **2.2. Chromosome Doubling**

To produce pure inbred lines for fundamental study and like also commercial crops, the doubled haploids approach was developed. According to the protocol for each species, the approach typically begins with the generation of HPs followed by chromosome duplication that may be spaced and overlapped in specific time. Production of HPs is frequently required for greater optimization and has delayed research and developments in

chromosomal doubling techniques (Hooghvorst and Nogues, 2021). Haploid plantlets can be transformed to diploids through application of different chemical solutions including commonly known as colchicine solution that depending on *in vivo* or *in vitro* explants submerged in chromosome duplication chemicals as colchicine concentration at a specific time. Frequently, treatments of low colchicine concentration in short-term have given the pivotal results in a view of duplication and survival rates. For instance, (Lim et al. 2008; Solmaz et al. 2011b), described that *in vivo* shoot tips submerged into colchicine concentration of 5000 mgL<sup>-1</sup> in two hours or *in vitro* single nodal/micro-cuttings explants into 500 mg L<sup>-1</sup> of colchicine for twelve hours are an efficient protocol which can be applied in chromosome doubling processes for cucurbit plants. Besides, various chemicals for chromosome doubling known as reagents such as oryzalin, trifluralin, amiprofos-methyl are available on markets and are being successful application in many crops (Kato, 2001; Bae et al. 2020; Garcia-Lozano et al. 2021).

The current protocol applied 50 mgL<sup>-1</sup> of oryzalin for 18 hrs (Ebrahimzadeh et al. 2018). Prior to antimetabolic application, roots and stems of plantlets have been cut into pieces of 3cm of length and have been incubated within a solution of antimetabolic reagent in 5 hrs at 25°C under sterile conditions on a shaker at 120 rpm speed. Furthermore, the results demonstrated that increasing the concentration of antimetabolic reagent also raise the rate of plantlet mortality to the 91.11% in the 1000 mg/l of colchicine concentration. In the other hand, the mid-range concentration experiment of oryzalin for 2.5 mg/l concentration demonstrated the high number of mortalities, at the rate of 34.78% (Hooghvorst et al. 2018; Hooghvorst and Nogues, 2021). Currently, many and various techniques such as morphological observations, marker-assisted selection, chromosome

counting, flow cytometry, pollen counts, cytological analysis, and seed germination are useful for ploidy level identification in cucurbit species (Dong et al. 2016; Sari and Solmaz 2021). The use of colchicine (0.1%) of haploid chromosome doubling in cucumber haploid shoot apical meristems that have immersed in colchicine solution for 1 hour. The ploidy levels for the plant were carried out utilizing flow cytometry after 30 days of culturing (Deng et al. 2020).

## CONCLUSION

Most of researchers have been reported that haploid plant production technology has various factors (obstacles) that affect the increase of haploid plant formation level in *Cucurbitaceae* family including culture conditions, nutrient media, development stages, pre-treatment conditions, heat shock application, and genotypes of donor plants; for the case of genotype most cucurbit species are recalcitrant. According to many studies, it is very important to select appropriate genotypes for haploid plant stimulation by different techniques. Further studies are needed for efficient protocols in almost cucurbit haploid induction through gynogenesis and androgenesis for supporting and reducing the costs that have been found in using irradiated pollen methods. The genotype independent new protocols should be designed and developed via different research by improving existing ones particularly on modification of culture media, heat shock applications and cold pre-treatments.

**ABBREVIATIONS:** **2, 4-D:** 2-Dichlorophenoxyacetic Acid; **DH:** Doubled Haploid; **GA3:** Gibberellic Acid; **MC:** Microspore Culture; **MS:** Murashige and Skoog; **NAA:** a-Naphthaleneacetic Acid; **IBA:** Indole-3-Butyric Acid; **KIN:** Kinetin; **PGR:** Plant Growth Regulator; **PUT:** Putrescine; **SPD:** Spermidine; **TDZ:** Thidiazuron; **AC:** Anther culture; **DH:**

Double Haploid; **HP:** Haploid plantlet; **OC:** Ovary/Ovule culture; **TP:** tetraploid plantlet; **CP:** Chimeric plantlet;  $\gamma$  (**Co<sup>60</sup>**): Gamma ray source (Cobalt 60); **LB:** light box containing a small white fluorescent lamp; **BA:** N6-Benzylaminopurine; **LMC:** liquid medium culture; **MI:** morphology identification; **OI:** opened individually; **XR:** X-ray radiography.

## REFERENCES

- Abdollahi, M. R., Darbandi, M., Hamidvand, Y. and Majdi, M. (2015). The influence of phytohormones, wheat ovary co-culture, and temperature stress on anther culture response of watermelon (*Citrullus lanatus* L.), Brazilian Journal of Botany, 38(3), 447-456.
- Abdollahi, M. R., Najafi, S., Sarikhani H., and Moosavi, S. S. (2016). Induction and development of anther-derived gametic embryos in cucumber (*Cucumis sativus* L.) by optimizing the macronutrient and agar concentrations in culture medium, Turkish Journal of Biology, 40(3): 571-579.
- Akbaş, F. C. and Solmaz, İ. (2019). Obtention of haploid plant in *Citrullus lanatus* var. *lanatus* and *Citrullus lanatus* var. *citroides* species by anther culture method. International Journal of Environmental Research and Technology, 2(3), 25-36.
- Asadi, A., Zebarjadi, A., Abdollahi M. R. and Seguí-Simarro, J. M. (2018). Assessment of different anther culture approaches to produce doubled haploids in cucumber (*Cucumis sativus* L.), Euphytica, 214(11): 216.
- Asadi, A., Zebarjadi, A. and Abdollahi, M. R. (2019). The influence of plant regulators on callus and embryo induction in cucumber (*Cucumis sativus* L.) anther culture, Iranian Journal of Horticultural Science, 49(4), 859-868.
- Asadi, A. and Seguí-Simarro, J. M. (2021). Production of doubled haploid plants in cucumber (*Cucumis sativus* L.) through anther culture, In Doubled Haploid Technology, (pp. 71-85). Humana, New York, NY. [https://doi.org/10.1007/978-1-0716-1331-3\\_4](https://doi.org/10.1007/978-1-0716-1331-3_4)
- Amirian, R., Hojati, Z. and Azadi, P. (2020). Male flower induction significantly affects androgenesis in cucumber (*Cucumis sativus* L.), The Journal of Horticultural Science and Biotechnology, 95(2), 183-191.
- Bae, S. J., Islam, M. M., Kim, H. Y. and Lim, K. B. (2020). Induction of Tetraploidy in watermelon with oryzalin treatments, Horticultural Science and Technology, <https://doi.org/10.7235/HORT.20200037>
- Baktemur, G; Yücel, N. K., Taşkın, H., Çömlekçiöğlü, S., and Büyükalaca, S. (2014). Effects of different genotypes and gamma ray doses on haploidization using irradiated pollen technique in squash, Turkish Journal of Biology: Vol. 38: No. 3.
- Baktemur, G., Keleş, D., Kara, E., Yıldız, S., and Taşkın, H. (2022). Effects of genotype and nutrient medium on obtaining haploid plants through ovary culture in cucumber. Molecular Biology Reports, 1-8.
- Chee, R. P., Leskovar, D. I., Cantliffe, D. J. (1992). Optimizing embryogenic callus and embryo growth of a synthetic seed system for sweet potato by varying media nutrient concentrations, J Amer Soc Hortic Sci 117: 663–667
- Claveria, E., Garcia-Mas, J., and Dolcet-Sanjuan, R. (2005). Optimization of cucumber doubled haploid line production using in vitro rescue of in vivo induced parthenogenic embryos, J Am Soc Hort Sci 130(4):555–560
- Dal, B., Sari, N., and Solmaz, I. (2016). Effect of different irradiation sources and doses on haploid embryoinduction in Altınbas (*Cucumis melo* L. var.

- inodorus*) melons. Turkish Journal of Agriculture and Forestry, 40(4), 552-559.
- Deng, Y., Tang, B., Zhou, X., Fu, W., Tao, L., Zhang, L. and Chen, J. (2020). Direct regeneration of haploid or doubled haploid plantlets in cucumber (*Cucumis sativus* L.) through ovary culture, Plant Cell, Tissue and Organ Culture, 253-268.
- de Oliveira, F. I. C., Carvalho, A. V. F., de Aragão, F. A. S. and de Carvalho, A. C. P. P. (2022). Growth regulators in the induction of calli in anthers of the Goldex hybrid yellow melon, Revista Agro@mbiente On-line, 16, 14-14. <https://doi.org/10.18227/1982-8470ragro.v16i0.7224>
- Dhillon, N. P., Sanguansil, S. Srimat, S. Cheng, H. Lin, C. Ramasamy, S et al. (2016). Status of cucurbit breeding at AVRDC-the World Vegetable Center, *Cucurbitaceae* 2016, XIth Eucarpia Meeting on Cucurbit Genetics & Breeding, July 24-28, 2016, Warsaw, Poland, Cucurbitaceae 2016 Organizing Committee.
- Dhillon, N. P., Laenoi, S., Srimat, S., Pruangwitayakun, S., Mallappa, A., Kapur, A., and Hanson, P. (2020). Sustainable cucurbit breeding and production in asia using public-private partnerships by the world vegetable center, Agronomy, 10(8), 1171.
- Diao, W. P., Jia, Y. Y., Song, H., Zhang, X. Q., Lou, Q. F et al. (2009). Efficient embryo induction in cucumber ovary culture and homozygous identification of the regenerants using SSR markers, Scientia Horticulturae, **119**(3): 246-251.
- Domblides, E., Shmykova, N., Khimich, G., Korotseva, I., Kan, L., Domblides, A. and Soldatenko, A. (2019). Production of doubled haploid plants of *Cucurbitaceae* family crops through unpollinated ovule culture in vitro, In VI International Symposium on Cucurbits, 1294 (pp. 19-28).
- Dong, Y.-Q., Zhao, W.-X., Li, X.-H., Liu, X.-C., Gao, N.-N et al. (2016). Androgenesis, gynogenesis, and parthenogenesis haploids in cucurbit species, Plant cell reports, **35**(10): 1991-2019.
- Ebrahimzadeh, H., Lotfi, M., Azizinia, S., and Ghanavati, F. (2013). Production of haploids in Cucurbita pepo L. through parthenogenesis induced by gamma-irradiated pollen, Crop Biotechnology, 3(4): 99-108.
- Ebrahimzadeh, H., M. Lotfi, and Sadat-Hosseini, M. J. (2021). Parthenogenetic haploid plant production in styrian pumpkin by gamma irradiated pollen, International Journal of Horticultural Science and Technology, 8(3): 285-294.
- El-Maksoud, A., El-Gendy, S. E. and El-Kady, M. M. (2009). Genotypes and genotype× medium composition interaction effects on androgenetic haploid production in cucumber (*Cucumis sativus* L.), Journal of Agricultural Chemistry and Biotechnology, 34(11), 10305-10312.
- Gałązka, J. and Niemirowicz-Szczytt, K. J. (2013). Review of research on haploid production in cucumber and other cucurbits, Folia Horticulturae, **25**(1): 67-78.
- Garcia-Lozano, M., Natarajan, P., Levi, A., Katam, R., Lopez-Ortiz, C., Nimmakayala, P. and Reddy, U. K. (2021). Altered chromatin conformation

- and transcriptional regulation in watermelon following genome doubling. *The Plant Journal*, 106(3), 588-600.
- Germana, M. A. (2011). Anther culture for haploid and doubled haploid production, *Plant Cell, Tissue and Organ Culture*, 104(3): 283-300.
- Ghanbari, S. and Golabadi, M. (2020). Effect of temperature pre-treatment and light on embryogenesis and callus induction in anther culture of cucumber, *Journal of Plant Research (Iranian Journal of Biology)*, 33(3), 542-552.
- Godbole, M., And Murthy, H. N. (2012). Parthenogenetic haploid plants using gamma irradiated pollen in snapmelon (*Cucumis Melo Var. Momordica*). *Tissue and Organ Culture* **109**(1): 167-170.
- Golabadi, M., Ghanbari, S., Keighobadi, K. and Ercisli, S. (2017). Embryo and callus induction by different factors in ovary culture of cucumber, *Journal of Applied Botany and Food Quality*, 90: 68-75.
- Gürsoy, I., Solmaz, I., Deliboran, S. and Sari, N. (2012). In vitro ovule and ovary culture in watermelon, *Proceedings of The Xth Eucarpia Meeting on Genetics and Breeding of Cucurbitaceae*, Antalya, Turkey, 15-18 October, 2012.
- Hamidvand, Y., Abdollahi, M. R., Chaichi M., and Moosavi, S. (2013). The effect of plant growth regulators on callogenesis and gametic embryogenesis from anther culture of cucumber (*Cucumis sativus* L.), *International Journal of Agriculture Crop Sciences*, 5 (10): 1089-1095.
- Hooghvorst, I., Ramos-Fuentes, E., Lopez-Cristoffanini, C., Ortega, M., Vidal, R et al. (2018). Antimitotic and hormone effects on green double haploid plant production through anther culture of mediterranean *Japonica* rice, *Plant Cell Tissue and Organ Culture*, 134(2): 205-215.
- Hooghvorst, I., Torrico, O., Hooghvorst, S. and Nogués, S. (2020). In situ parthenogenetic doubled haploid production in melon “Piel de Sapo” for breeding purposes, *Frontiers in Plant Science*, 11, 378.
- Hooghvorst, I. and Nogués, S. (2021). Chromosome doubling methods in doubled haploid and haploid inducer-mediated genome editing systems in major crops, *Plant Cell Reports*, 40(2), 255-270.
- d’Hooghvorst, I., Torrico, O. and Nogués, S. (2021). Doubled haploid parthenogenetic production of melon ‘Piel de Sapo’, In *Doubled Haploid Technology*, (pp. 87-95). Humana, New York, NY.
- Ivanova, Z. (2020). Obtaining of melon plant-regenerants via pollination with irradiated pollen, *Сборник доклади*, 28.
- Koksal, N., Yetisir, H., Sari, N., and Abak, K. (2001). Comparison of different in vivo methods for chromosome duplication in muskmelon (*Cucumis melo* L.), In *II International Symposium on Cucurbits* 588 (pp. 293-298). <https://doi.org/10.17660/ActaHortic.2002.588.46>
- Kumar, H. A., Murthy, H. N. and Paek, K. Y. (2003). Embryogenesis and plant regeneration from anther cultures of *Cucumis sativus* L, *Scientia horticulturae*, 98(3), 213-222.
- Kumar, H. G. A., and Murthy H. N. (2004). Effect of sugars and amino acids on androgenesis of *Cucumis sativus* L. *Plant Cell Tissue And Organ Culture* 78(3): 201-208.

- Kurtar, E.S., Sari, N., Abak, K. (2002). Obtention of haploid embryos and plants through irradiated pollen technique in squash (*Cucurbita pepo* L.), *Euphytica* 127(3):335–344
- Kurtar, E. S., Seymen, M., Çetin, A. N. and Türkmen, Ö. (2021b). Dihaploidization in promising summer squash genotypes (*Cucurbita Pepo* L.) via irradiated pollen technique, *Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi*, 31(1): 42-51.
- Kurtar, E. S., and Seymen, M. (2021a). Gynogenesis in *Cucurbita* Species, In *Doubled Haploid Technology*, (pp. 123-133). Humana, New York, NY.
- Kurtar, E. S. and Seymen, M. (2021c). Anther Culture in *Cucurbita* Species, In *Doubled Haploid Technology*, (pp. 111-121). Humana, New York, NY.
- Li, J., Si, S., Cheng, J., Li, J. and Liu J. J. (2013). Thidiazuron and silver nitrate enhanced gynogenesis of unfertilized ovule cultures of *Cucumis Sativus* L, *Biologia Plantarum*, 57(1): 164-168.
- Lotfi, M., Salehi, S., and Pitrat, M. (2008). Detection of cucumber parthenogenic haploid embryos by floating of immature seeds in liquid medium, *Cucurbitaceae* 2008, Proceedings of the IXth EUCARPIA meeting on genetics and breeding of *Cucurbitaceae* (Pitrat M, ed), INRA, Avignon (France), May 21-24th, 2008
- Malik, A. A., L., Cui, S., Zhang, X. and Chen, J. F. (2011). Efficiency of SSR markers for determining the origin of melon plantlets derived through unfertilized ovary culture, *Horticultural Science*, 38(1): 27-34.
- Mandoulakani, B. A., Rahmanpour, S., Shaaf, S., Khoei, S. G., Rastgou, M. and Rafezi, R. (2015). Towards the identification of retrotransposon-based and ISSR molecular markers associated with populations resistant to *Zymv* in melon, *South African Journal of Botany*, 100: 141-147.
- Min, Z., Li, H., Zou, T., Tong, L., Cheng, J., and Sun, X. J. (2016). Studies of in vitro culture and plant regeneration of unfertilized ovary of pumpkin, *Chinese Bulletin of Botany*, 51(1): 74.
- Mohamed, M., and Refaei, E. J. (2004). Enhanced haploids regeneration in anther culture of summer squash (*Curcurbita pepo* L.), *Report-Cucurbit Genetics Cooperative*, 27: 57.
- Moqbeli, E., Peyvast, G., Hamidoghli, Y. and Olfati, J. A. (2013). In vitro cucumber haploid line generation in several new cultivars, *Aspac J Mol Biol Biotechnol*, 21(1): 18-25.
- Murashige, T. and Skoog, F., J. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures, *Physiologia Plantarum*, 15(3): P. 473-497.
- Nguyen, T. T. V. and Chen, J. (2012). Cucumber (*Cucumis sativus* L.) anther culture, *China Cucurbits and Vegetables*, 25(2), 1-5.
- Nguyen, M. L., Ta, T. H. T., Huyen, T. N. B. T. and Voronina, A. V. (2019). Anther-derived callus formation in bitter melon (*Momordica charantia* L.) as influenced by microspore development stage and medium composition, *Sel'skokhozyaistvennaya Biologiya*, 54(1), 140-148.

- Ozsan, T., Gozen, V., and Onus, A. (2017). Cucumber gynogenesis: effects of 8 different media on embryo and plant formation, *International J. of Agriculture Innovations Research*, 6(2): 419-422.
- Salehi, B., Quispe, C., Sharifi-Rad, J., Giri, L., Suyal, R., Jugran, A. K., ...and Zam, W. (2021). Antioxidant potential of family Cucurbitaceae with special emphasis on *Cucurbita* genus: A key to alleviate oxidative stress-mediated disorders, *Phytotherapy Research*, 35(7), 3533-3557.
- Sang, T. (2002). Utility of low-copy nuclear gene sequences in plant phylogenetics, *Critical Reviews in Biochemistry and Molecular Biology*, 37, 121-147.
- Sari, N., Abak, K., Pitrat, M., Dumas de Vault, R. (1992). Induction of parthenogenetic haploid embryos and plant obtention in melon (*Cucumis melo* L.var.*inodorus* Naud and *C.melo* L.var.*reticulatus* Naud), *Doga Turkish Journal of Agric. and Forestry*, 16, 302-314.
- Sari, N., Abak, K., Pitrat, M., Rode, J. C., and Dumas de Vault, R. (1994). Induction of parthenogenetic haploid embryos after pollination by irradiated pollen in watermelon, *HortScience* 29: 1189-1190.
- Sari, N., Abak, K., and Pitrat, M. (1999). Comparison of ploidy level screening methods in watermelon: *Citrullus lanatus* (Thunb.) Matsum. and Nakai, *Sci Hort* 82(3):265-277
- Sari, N., and Solmaz, I. (2021). Doubled haploid production in watermelon, In *Doubled Haploid Technology*, (pp. 97-110). Humana, New York, NY.
- Seguí-Simarro, J. and Nuez, F. (2004). Androgenesis induction from tomato anther cultures: callus characterization. V *International Symposium on In Vitro Culture and Horticultural Breeding* 725.
- Segui-Simarro, J. M. (Ed.). (2021). *Doubled haploid technology: Volume 3: Emerging Tools, Cucurbits, Trees, Other Species* Springer US.
- Solmaz, İ., Sari, N., Caymaz, G., GURSOY, I., GOCMEN, M., GOKSEVEN, A., and AYDIN, E. (2011a). Karpuzda haploid embriyo uyartımı ve bitki elde edilmesi üzerine farklı çeşit ve *in vitro* kültür ortamlarının etkisi, *Türkiye VI. Ulusal Bahçe Bitkileri Kongresi* (04-08 Ekim 2011 Şanlıurfa ).
- Solmaz, İ., Sari, N., Gürsoy, I., and Kasapoğlu, S. (2011b). Comparison of in vivo and in vitro Colchicine Application for Production of Dihaploid 'Kirkagac'and 'Yuva Hasanbey'Melons, *African Journal of Biotechnology*, 10(70), 15717-15724.
- Song, H., Lou, Q. F., Luo, X. D., Wolukau, J. N., Diao, W. P., Qian, C. T. and Chen, J. F. (2007). Regeneration of doubled haploid plants by androgenesis of cucumber (*Cucumis sativus* L.), *Plant Cell, Tissue and Organ Culture*, 90(3), 245-254.
- Suprunova, T. and Shmykova, N. (2008). In vitro induction of haploid plants in unpollinated ovules, anther and microspore culture of *Cucumis sativus* L., In *Cucurbitaceae* 2008. Proceedings of the IXth EUCARPIA meeting on genetics and breeding of *Cucurbitaceae*, Avignon, France, 21-24 May 2008 (pp. 371-374). Institut National de la Recherche Agronomique (INRA).
- Taşkın, H., Kemal Yücel, N., Baktetur, G., Çömlekçioğlu, S. and Büyükalaca, S. (2013). Effects of different genotypes and gamma ray doses on

- haploidization with irradiated pollen technique in watermelon (*Citrullus lanatus* L.), Canadian journal of plant science, 93(6), 1165-1168.
- Weng, Y., Z. J. G. Sun, F. (2011). Major cucurbit crops. Genetics and genomics breeding of cucurbits: CRC Press, USA 1-16.
- Xie, B., Wang, X. F., and Fan, Z. C (2006). Improved conditions of in vitro culture of unpollinated ovules and production of embryonary sac plants in summer squash (*Cucurbita pepo* L.), Sci Agric Sin 39(1):132-138
- Xu, Z., and Chang, L. (2017). *Cucurbitaceae*, In Identification and Control of Common Weeds: Volume 3 (pp. 417-432). Springer, Singapore.
- Yıldız, Ç. and Solmaz, İ. (2020). Karpuz genetik kaynaklarında ovül-ovaryum kültürü yöntemiyle haploid bitki elde edilmesi, Çukurova Tarım ve Gıda Bilimleri Dergisi, 35(1): 35-42.
- Yue, W., Yiqin, G., Bo, D. and Tonghui, R. (2005). Induction of watermelon anther callus, Hubei Agricultural Sciences, (5), 93-95.
- Zhan, Y., Chen, J. and Malik, A. A. (2009). Embryoid induction and plant regeneration of cucumber (*Cucumis sativus* L.) through microspore culture, Acta Horticulturae Sinica, 36(2), 221-226
- Zhang, X., Su, H., Yang, J., Feng, L., Li, Z., and Zhao, G. (2019a). Population genetic structure, migration, and polyploidy origin of a medicinal species *Gynostemma pentaphyllum* (*Cucurbitaceae*), Ecology and Evolution, 9 (19), 11145-11170.
- Zhang, N., Bao, Y., Xie, Z., Huang, X., Sun, Y., Feng, G., ... and Tang, M. (2019b). Efficient characterization of tetraploid watermelon, Plants, 8(10), 419.
- Zheng, Y., Wu, S., Bai, Y., Sun, H., Jiao, C., Guo, S et al. (2019). Cucurbit Genomics Database (CuGenDB): a central portal for comparative and functional genomics of cucurbit crops, Nucleic acids research, 47(D1): D1128-D1136.
- Zhu, Y. Sun, D., Yun, D., An, G., Li, W et al. (2020). Comparative transcriptome analysis of the effect of different heat shock periods on the unfertilized ovule in watermelon (*Citrullus lanatus* L.), Journal of Integrative Agriculture, 19(2): 528-540.
- Zhu, Y., Sun, Z., Sun, D., Deng, Y., Wang, Z. and Liu, J. (2010). Advances of watermelon anther culture technology, China Cucurbits and Vegetables, 23(1), 28-31.
- Zhu, Y., Sun, D., Deng, Y., An, G., Li, W. and Liu, J. (2012). Effects of preliminary treatment on watermelon anther culture callus induction, China Cucurbits and Vegetables, 25(5), 17-19.
- Zhu, Y., Sun, D., Deng, Y., Li, W., An, G., Si, W. and Liu, J. (2019). Regeneration of double haploid plants from unpollinated ovary cultures of watermelon. <https://assets.researchsquare.com/files/rs-4797/v1/72077673-eab9-474f-9769-0873bf11b52c.pdf?c=1631827131> (Accessed on 20 June 2022).
- Zou, T., Song, H., Chu, X., Tong, L., Liang, S., Gong, S., Yang, H., and Sun, X. (2020). Efficient induction of gynogenesis through unfertilized ovary culture with winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.), Scientia Horticulturae, 264, 109-152.

- Zhu, Y., Liu, J., Deng, Y., Li, W., An, G., and Sun, D. (2015). Effects of different culture factors on callus induction of watermelon anther, *Journal of Henan Agricultural Sciences*, 44(12), 104-111.
- Zou, T., Song, H., Chu, X., Tong, L., Liang, S., Gong, S. ... and Sun, X. (2020). Efficient induction of gynogenesis through unfertilized ovary culture with winter squash (*Cucurbita maxima Duch.*) and pumpkin (*Cucurbita moschata Duch.*), *Scientia Horticulturae*, 264, 109152.



## CHAPTER 3

# INVESTIGATION OF THE TEMPERATURE CHANGE OF SAND DRIED AT DIFFERENT TEMPERATURES IN A PILOT TYPE FLUIDIZED BED DRYER

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## 1. INTRODUCTION

Drying is the process of removing water or other liquids from gases, liquids, and solids. However, the most common use of the concept of drying is the process of removing water or volatile substances from solid materials by thermal methods (Güngör and Özbalta, 2009).

Drying is a complicated process that involves simultaneous heat and mass transfer. Water molecule transport has a close relationship with the drying process, including molecular diffusion, such as capillary motion, liquid diffusion, vapor diffusion, and hydrodynamics (Çelen et al., 2017).

Industrial raw materials include many different products due to their formation and usage areas, and the products included in this scope have a high importance in Turkish mining and export of mineral products. In this context, feldspar, perlite and pumice, magnesite, bentonite, trona, cement raw materials, sepiolite, ceramic clays and kaolin, quartz, quartz sand and quartzite and phosphate have been identified as important raw materials among industrial raw materials (Turkish Republic Ministry of Development, 2015).

Silica (quartz) sand is abundant in the earth's crust. The main areas of use are the glass industry, the casting industry, the refractory industry, chemistry, filtration, and construction industries (Kurşun and İpekoğlu, 1995).

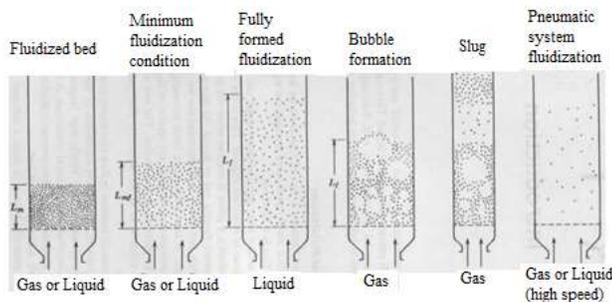
The literature introduces a wide range of industrial dryers for materials with various qualities. Some of these dryers are fluidized bed dryers (Chuwattanakul et al., 2022), spray dryers (Baker and McKenzie, 2005), vacuum dryer (Mella et al., 2022), microwave-solar dryers (Çelen and Arda, 2019), infrared dryers (Ning et al., 2015), microwave dryers (Köse Tınmaz et al., 2019).

Fluidized bed dryers can be divided into horizontal and vertical types according to the location of the fluidized bed drying chamber. Fluid bed dryers have the advantages of high product quality, short drying time, simple

construction, and low procurement and maintenance costs (Yahya et al., 2022). Also, one of the most significant benefits of these dryers is that adequate mixing takes place during the drying process, increasing drying efficiency (Nabizadeh et al., 2020). In addition, they can be used to dry wet granular and granular materials such as agricultural (rice, wheat, soybean, and corn), pharmaceutical, chemical, food, herbal, and heat-sensitive products (Yahya et al., 2022).

A typical fluidized bed drying system consists of a gas blower, a heater, a fluidized bed column, and gas cleaning systems such as cyclones, bag filters, precipitators, and scrubbers. Sometimes the off-gas is partially recycled to save energy (Mujumdar, 2006).

The conditions that occur in the bed according to the air velocity in fluidized bed dryers are shown in Figure 1. When air is supplied to the system faster than the ideal fluidization rate, the bubbles increase, causing large voids and violent boiling in the dryer. The system in which this type of boiling is applied is called a turbulent fluidized bed (Erbaş, 2007).



**Figure 1:** Bubbles that may form in the fluidized bed (Kunii and Levenspiel, 1977)

In the fluidized bed dryer, the wet material is loaded into the drying chamber of the batch fluidized bed dryer before the drying air starts to circulate. After the material is completely dry, it is removed from the drying

chamber. In this study, the drying behavior of silica sand with three different temperatures given from the bottom was investigated.

## **2. MATERIALS AND METHOD**

### **2.1. Silica Sand**

The silica sand used in this research was obtained from a company that produces and distributes cement, ready-mixed concrete, and aggregates. The silica sand obtained from the rocks is extracted with an average of 10% moisture. In order for it to be used in concrete production, the product moisture needs to be below 0.05% (Figure 2).



**Figure 2:** Silica sand sample

### **2.2. Sieve and Moisture Analyzer**

It is aimed at determining the grain size of silica sand used in drying experiments with the sieve analysis method. In this study, the particle sizes of the silica sand sample were determined with the sieve analyzer in the laboratory of Asos Proses Makina (Figure 3a). The moisture values of the samples taken at the beginning and during the experiments of the silica sand used in the drying experiments were measured with the RADWAG brand moisture analyzer (Figure 3b).



**Figure 3:** a) Sieve analyzer, b) Moisture Analyzer

### 2.3. Density Test Apparatus

In this study, the bulk density of the product was measured with the bulk density device and balance in the laboratory of Asos Process Engineering (Figure 4).

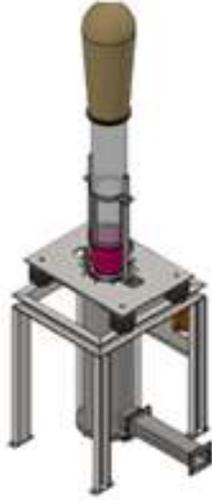


**Figure 4:** Bulk density meter and balance

### 2.4. Vertical Type Fluid Bed Dryer

The vertical type fluidized bed dryer used in the experiment is a stainless steel dryer with a diameter of 108 mm, a product chamber length of 151 mm, and a total height of 853 mm. There is a perforated stainless steel

sheet with 2 mm diameter holes inside. The drying system is a computer-controlled system (Figure 5).



**Figure 5:** Pilot vertical type fluidized bed dryer

## **2.5. Method**

### **2.5.1. Test Parameters and Measurement**

This study was carried out with a moist silica sand sample obtained from a construction company in the laboratory of Asos Proses Makina factory. Among the factors affecting the drying process in the drying experiment, temperature, bed height, and particle size parameters were investigated. During the experiments carried out, the product temperature and product moisture values at the end of the 3rd, 6th, 9th, and 12th minutes were measured. The product temperature was measured with a thermometer during sampling. The product moisture of the product samples taken at the specified minutes was measured with a moisture analyzer (Şengül, 2022).

The drying air temperature used in the experiments was determined as 90°C, 120 °C, and 150 °C, and the experiments were carried out at these

temperatures. The drying experiments of the silica sand used in the study were carried out at 150 mm bed height. In order to determine the particle size of the silica sand, it was dried in an oven at 105 °C for 24 hours and subjected to sieve analysis. According to the analysis results, the accepted particle size was 910 µm.

Bulk density is the bulk solid mass of interparticle voids that occupies a unit volume of a bed. In order to determine the particle size, the free bulk density was measured with the sample taken from the sand, which was dried in an oven at 105°C for 24 hours and provided moist (Table 1).

**Table 1.** Silica sand free bulk density

<b>Weight (g)</b>	<b>Volume (ml)</b>	<b>Density (g/ml)</b>
139.53	100	1.3953
139.05	100	1.3905
140.95	100	1.4095
Average:		1.3984

In order to measure the initial moisture values of silica sand, which was supplied in three different sizes and kept in an airtight manner, separate samples were taken and three measurements were made in the moisture analyzer. According to the values obtained, the average initial moisture values of the samples were determined as 9.57%.

### **3. RESEARCH FINDINGS**

#### **3.1. Moisture Change Analysis**

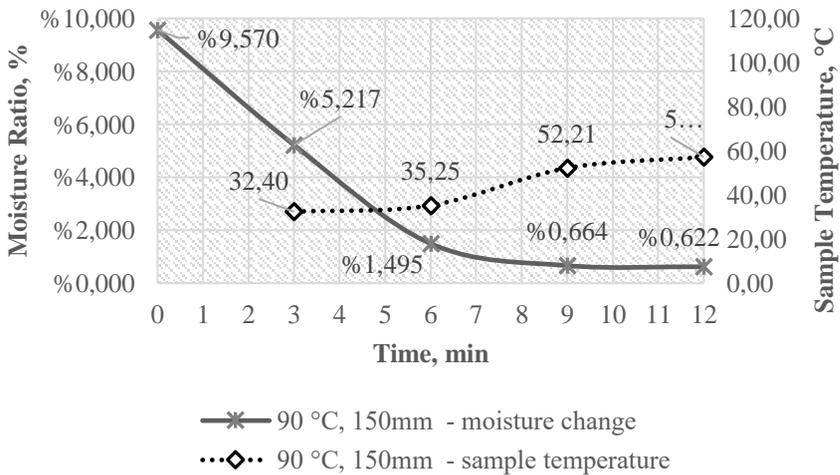
In Experiment 1, the values of which are given in Table 2, silica sand with an initial moisture of 9.57%, a bed height of 150 mm, and a wet weight of 2718 g was subjected to drying at 90 °C air temperature for 12 minutes. The experiments were carried out with vibration to provide fluidization to the sand. At the 3rd minute of the sample, the product temperature was measured

at 32.40 °C and the product moisture was 5.217%. The product temperature at the 6th minute was 35.25 °C and the product moisture was 1.495%. The product temperature at the 9th minute was 52.21 °C and the product moisture was 0.664%. At the 12th minute, the product temperature was 57.18 °C and the product moisture was 0.622% (Figure 6).

**Table 2.** Experiment (Exp)-1,  $dp_1= 0.000910$  m,  $h= 150$  mm,  $T= 90$  °C drying table

Exp	PS (m)	BH (mm)	DT (°C)	IM (%)	DT (min)	PT (°C)	PM (%)
Exp-1	0,000910	150	90	9,57	3	32,40	5,217
Exp-1	0,000910	150	90	9,57	6	35,25	1,495
Exp-1	0,000910	150	90	9,57	9	52,21	0,664
Exp-1	0,000910	150	90	9,57	12	57,18	0,622

PS: Particle Size, BH. Bed Height, DT: Drying Temperature, IM: Initial Moisture, DT: Drying Time, PT: Product Temperature, PM: Product Moisture



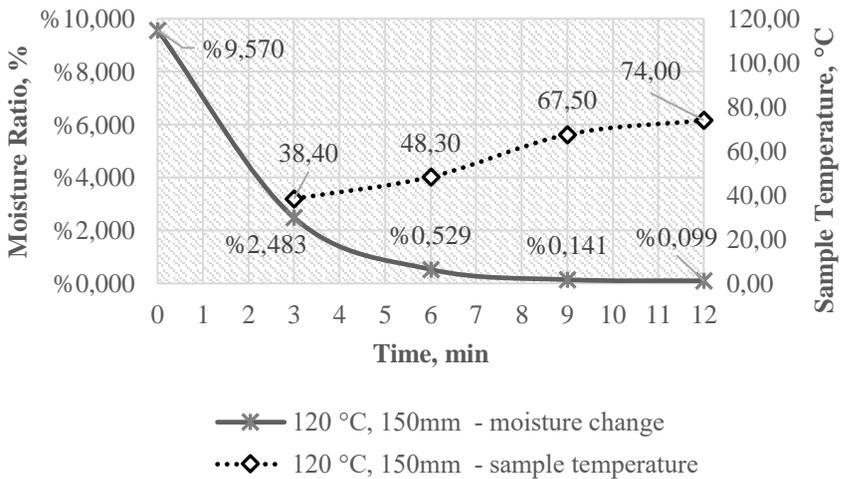
**Figure 6:** Experiment -1,  $dp_1= 0.000910$  m,  $h= 150$  mm,  $T= 90$  °C product moisture and temperature change

In Experiment 2, the values of which are given in Table 3, silica sand with an initial moisture of 9.57%, a bed height of 150 mm, and a wet weight of 2718 g was subjected to drying at 90 °C air temperature for 12 minutes.

The experiments were carried out with vibration to provide fluidization to the sand. At the 3rd minute of the sample, the product temperature was measured at 38.40 °C and the product moisture was 2.483%. The product temperature at the 6th minute was 48.40 °C and the product moisture was 0.529%. The product temperature at the 9th minute was 67.50 °C and the product moisture was 0.141%. At the 12th minute, the product temperature was 74.00 °C and the product moisture was 0.099% (Figure 7).

**Table 3.** Experiment-2,  $dp_1= 0.000910$  m,  $h= 150$  mm,  $T= 120$  °C drying table

Exp	PS (m)	BH (mm)	DT (°C)	IM (%)	DT (min)	PT (°C)	PM (%)
Exp-2	0,000910	150mm	120 °C	9,57%	3	38,40 °C	2,483%
Exp-2	0,000910	150mm	120 °C	9,57%	6	48,30 °C	0,529%
Exp-2	0,000910	150mm	120 °C	9,57%	9	67,50 °C	0,141%
Exp-2	0,000910	150mm	120 °C	9,57%	12	74,00 °C	0,099%

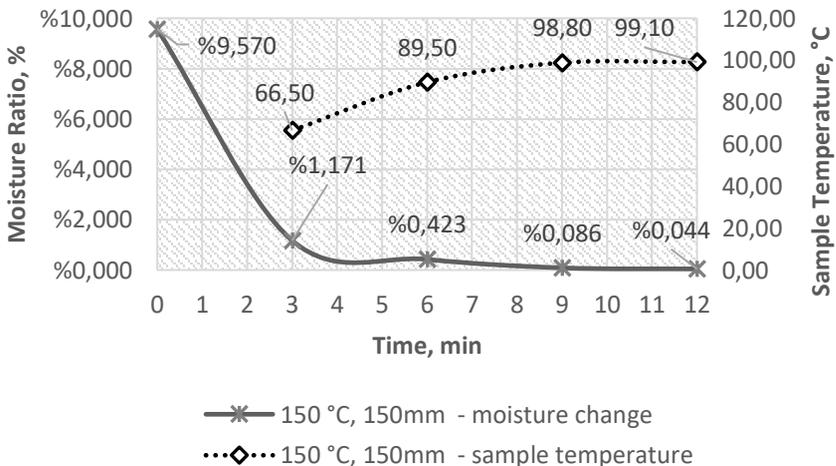


**Figure 7:** Experiment-2,  $dp_1= 0.000910$  m,  $h= 150$  mm,  $T= 120$  °C product moisture and temperature change

In Experiment 3, the values of which are given in Table 2.4, silica sand with an initial moisture of 9.57%, a bed height of 150 mm, and a wet weight of 2718 g was subjected to drying at 90 °C air temperature for 12 minutes. The experiments were carried out with vibration to provide fluidization to the sand. At the 3rd minute of the sample, the product temperature was measured at 66.50 °C and the product moisture was 1.171%. The product temperature at the 6th minute was 89.50 °C and the product moisture was 0.423%. The product temperature at the 9th minute was 98.80 °C and the product moisture was 0.086%. At the 12th minute, the product temperature was 99.10 °C and the product moisture was 0.044% (Figure 8).

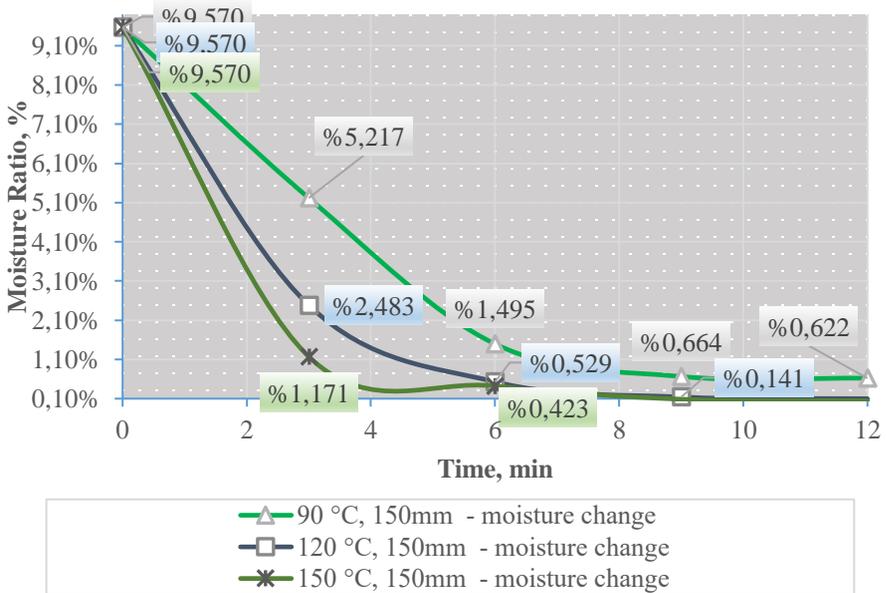
**Table 4.** Experiment-3,  $d_{p1}= 0.000910$  m,  $h= 150$  mm,  $T= 150$  °C drying table

Exp	PS (m)	BH (mm)	DT (°C)	IM (%)	DT (min)	PT (°C)	PM (%)
Exp-3	0,000910	150mm	150 °C	9,57%	3	66,50 °C	%1,171
Exp-3	0,000910	150mm	150 °C	9,57%	6	89,50 °C	%0,423
Exp-3	0,000910	150mm	150 °C	9,57%	9	98,80 °C	%0,086
Exp-3	0,000910	150mm	150 °C	9,57%	12	99,10 °C	%0,044



**Figure 8:** Experiment-3,  $d_{p1}= 0.000910$  m,  $h= 150$  mm,  $T= 150$  °C product moisture and temperature change

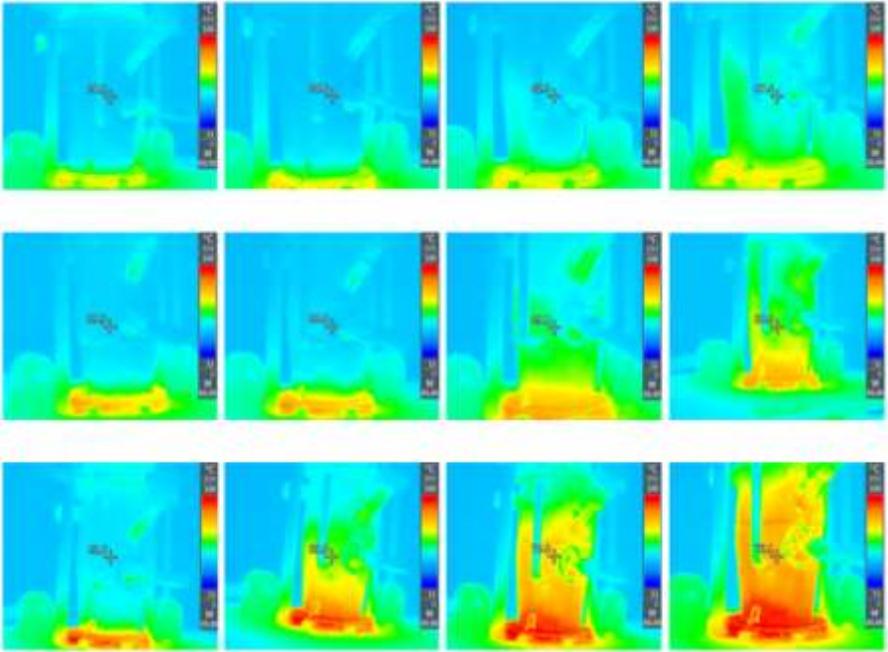
When figure 9 is evaluated, it is observed that the moisture of the product decreases over time and the temperature increases over time. The time-dependent graph of the moisture loss of the product according to the drying air temperature of silica sand with a diameter of 0.000910 m and a bed height of 150 mm is shown in Figure 9.



**Figure 9:**  $dp_1=0.000910$  m,  $h=150$ mm,  $90^{\circ}\text{C}$ - $120^{\circ}\text{C}$ - $150^{\circ}\text{C}$  product moisture change comparison chart

In all experiments, it was observed that the moisture content decreased as the temperature increased.

Figure 10 shows the thermal camera images during the drying of the silica sand sample with a  $dp_3=0.000910$  m bed height of 150 mm and an air temperature of  $90^{\circ}\text{C}$ ,  $120^{\circ}\text{C}$ , and  $150^{\circ}\text{C}$ , respectively, in a vertical type fluidized bed dryer for 12 minutes. In thermal analysis, the green parts in the chamber are the distribution of silica sand samples within the chamber that have started to dry. The red parts are the overheated parts. Since hot air is supplied to the dryer from the bottom, the bottom of the product is the hottest part.



**Figure 10:** Thermal camera images at 90 °C, 120 °C and 150 °C air temperatures

#### 4. CONCLUSION

In this study, the effects of drying of silica sand at three different drying temperatures (90 °C, 120 °C, and 150 °C) in a vertical fluidized bed dryer on parameters such as 0.000910 m particle size and 150 mm bed height were experimentally investigated. In all experiments, it was observed that the moisture decreased with temperature. In the analysis made with a thermal camera, it has been observed that drying at 150°C is effective and dries in the shortest time.

#### ACKNOWLEDGEMENTS

The authors thank Asos Process Engineering for making use of its laboratory.

## REFERENCES

- Baker, C.G.J. and McKenzie, K.A. (2005). Energy Consumption of Industrial Spray Dryers, *Drying Technology*. Vol. 23, No. 1, pp 365-386.
- Chuwattanakul, V., Wongchare, K., Pimsarn, M., Chokphoemphun, S., Chamoli, S. and Eiamsa-ard, S. (2022). Effect of conical air distributors on drying of peppercorns in a fluidized bed dryer: Prediction using an artificial neural network, *Case Studies in Thermal Engineering*, Vol. 36, pp 102188.
- Çelen, S. and Arda, S. O. (2019). Design Of Semisphere Solar-Microwave Hybrid Dryer And Drying Performance Of Zucchini, *Journal of Agricultural Science and Technology*, Vol. 21, No. 5, pp 1131-1143.
- Çelen, S., Haksever, A. and Moralar, A. (2017). The Effects of Microwave Energy to the Drying of Apple (Gala) Slices, *Karaelmas Science & Engineering Journal*, Vol. 7, No. 1, pp 228-236.
- Erbaş, O. (2007). Dolaşımli akışkan yatakta ısı transferi mekanizması ve bu mekanizmanın kuramsal ve deneysel analizi (Doktora Tezi), Gazi Üniversitesi Fen Bilimleri Enstitüsü, Ankara.
- Güngör, A. and Özbalta, N. (2009). Kurutmanın Temelleri ve Endüstriyel Kurutucular Kurs Notları. IX. Ulusal Tesisat Mühendisliği Kongresi. İzmir.
- Köse Tınmaz E., Çelen, S., Çelik S. Ö. (2019). Conventional and microwave drying of hydrocarbon cutting sludge, *Environmental Progress & Sustainable Energy*, Vol. 38, No. 4, pp 1-7.
- Kunii, D., & Levenspiel, O. (1991). Fluidization Engineering. Butterworth-Heinemann. ISBN:0-88275-542-0
- Kurşun, İ. and İpekoğlu, B. (1995). *Türkiye kuvars kumu potansiyeline genel bir bakış*. Endüstriyel Hammaddeler Sempozyumu. Nisan 21-22, İzmir, Türkiye (in Turkish).
- Mella, C., Vega-Galvez, A., Uribe, E., Pasten A., Mejias, N. And Quispe-Fuentes, I. (2022). Impact of vacuum drying on drying characteristics and functional properties of beetroot (Beta vulgaris), *Applied Food Research*, Vol. 2, pp 100120.
- Ministry of Development of the Republic of Turkey (2015). Onuncu Kalkınma Planı (2014-2018) Madencilik Politikaları Özel İhtisas Komisyonu Raporu. ISBN: 978-605-9041-27-0. Ankara: Yayın No: KB:2926- ÖİK:753 (Access link: [https://www.sbb.gov.tr/wp-content/uploads/2018/10/10\\_MadencilikPolitikalari.pdf](https://www.sbb.gov.tr/wp-content/uploads/2018/10/10_MadencilikPolitikalari.pdf)) (in turkish).
- Mujumdar, A.S. (Ed.). (2006). *Handbook of Industrial Drying* (3rd ed.). CRC Press.
- Nabizadeh, A., Hassanzadeh, H., Asadieraghi, M., Hassanpour, A., Moradi, D., Keshavarz, M. And Namin, M.H. (2020). A parametric study of the drying process of polypropylene particles in a pilot-scale fluidized bed dryer using Computational Fluid Dynamics, *Chemical Engineering Research and Design*, Vol. 156, pp 13–22.
- Ning, X., Lee, J. and Han, C. (2015). Drying characteristics and quality of red ginseng using far-infrared rays, *J Ginseng Res* Vol.39, pp 371-375.
- properties of beetroot (Beta vulgaris), *Applied Food Research*, Vol. 2, pp 100120.
- Yahya, M., Rachman, A. and Hasibuan, R. (2022). Performance analysis of solar-biomass hybrid heat pump batch-type horizontal fluidized bed dryer using multi-stage heat exchanger for paddy drying, *Energy*, Vol. 254, pp 124294.

## **CHAPTER 4**

### **PAST, PRESENT AND FUTURE OF FIELD AGRICULTURE: THE CASE OF MALKARA DISTRICT OF TEKİRDAĞ PROVINCE**

MSc. Fatih BÜYÜKFİLİZ<sup>1</sup>

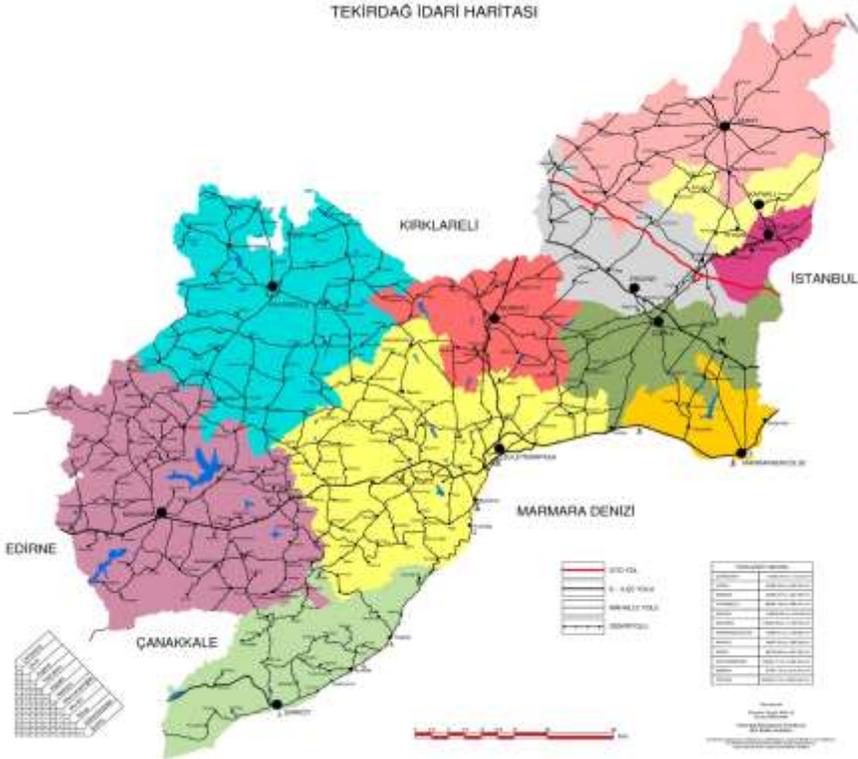
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## INTRODUCTION

Malkara district of Tekirdağ province, located in the Thrace region, has been home to agriculture for many years. The district is located 56 km from the center of Tekirdağ. Keşan is located in the west of the district, Uzunköprü in the northwest, Hayrabolu in the northeast, Şarköy in the southeast and Gelibolu in the south. Located between 40.54 north latitude and 26.52 east longitude in the world, the district has the largest land area in Tekirdağ province and its surface area is 1.225 km<sup>2</sup> [Fig. 1 (Anonymous, 2022a) and Fig. 2 (Anonymous, 2022b)].



**Figure 1:** Map of Tekirdağ province (Anonymous, 2022a).



Malkara district are stone-free, deep-structured and around neutral in terms of pH, the most obvious deficiency of the agricultural soils of the region is that they are getting poorer day by day in terms of organic matter and the amount of available Zn is insufficient (Bellitürk, 2011; Bellitürk, 2019). In this regard, soil analysis should be given importance especially in wheat and sunflower agriculture, which is intensively done in the region, and producers should be informed and even supported about the widespread use of organic and organo mineral fertilizers in addition to chemical fertilizers in fertilization programs (Bellitürk and Çelik, 2022).

**Table 1:** Tekirdağ province meteorological data (Anonymous, 2022d).

TEKİRDAĞ	January	February	March	April	May	June	July	August	September	October	November	December	Yearly
Measurement Period (1940-2021)													
Average Temperature (°C)	4.8	5.5	7.3	11.7	16.7	21.1	23.7	23.8	20.2	15.6	11.3	7.2	14.1
Average Maximum Temperature (°C)	8.1	9.0	11.1	15.7	20.6	25.3	28.0	28.2	24.5	19.5	14.8	10.4	17.9
Average Lowest Temperature (°C)	2.0	2.5	4.1	8.1	12.7	16.7	19.0	19.4	16.1	12.1	8.1	4.3	10.4
Average Sunbathing Time (hours)	2.7	3.3	4.2	5.8	7.3	8.6	9.4	8.5	6.8	4.6	3.2	2.5	5.6
Average Number of Rainy Days	10.76	10.35	11.65	8.24	8.41	7.82	3.24	1.59	5.35	9.41	8.88	11.12	96.8
Average Monthly Total Rainfall (mm)	69.5	54.6	53.9	41.1	37.6	38.7	24.2	15.4	33.4	61.4	73.2	80.5	583.5
Measurement Period (1940-2021)													
Highest Temperature (°C)	23.9	24.7	28.1	34.3	33.8	40.2	38.4	39.4	39.7	35.1	27.9	23.5	40.2
Lowest Temperature (°C)	-13.5	-13.3	-10.4	-1.2	2.7	8.6	10.9	11.0	3.7	-1.8	-7.8	-10.9	-13.5

In this study, the situation of agricultural production potential in Malkara district of Tekirdağ province, which is one of the most important centers of agriculture in Turkey, in the last five years has been examined and evaluated. In this compilation study, the last five years of plant and animal information belonging to the Malkara district of Tekirdağ province were evaluated according to the examination and scientific criteria.

## 2. AGRICULTURAL PRODUCTION IN MALKARA DISTRICT

### 2.1. Plant Production

Malkara district has been prominent with agriculture since pre-Republican times. Reasons such as the flatness of the surface, the favorable climatic conditions, the fertility of the soil, and the easy marketing of the cultivated products have brought this district to the forefront in the field of agriculture. Agriculture is carried out mainly in open field conditions in the region. There has been an increase in the cultivated agricultural areas in the district day by day. The data for the last five years of the cultivated areas in Malkara district are shown in Table 2 (TURKSTAT, 2022).

**Table 2:** Cultivated areas in Malkara district of Tekirdağ province.

Production Year	Cultivated Area (da)
2017	759.929,40
2018	759.233,40
2019	750.539,40
2020	750.589,50
2021	795.781,00

As seen in Table 2, a significant increase has been observed in the cultivated agricultural areas in recent years. It has been understood that the

main reason for this increase is the increase in the demand for food of the increasing population day by day and that production should be done in all agricultural areas that can be cultivated in order to meet this demand.

According to the data of the Ministry of Agriculture and Forestry in the district, the number of registered farmers has been increasing in recent years. The data regarding the number of farmers registered in the Farmer Registration System for the last five years in Malkara district are shown in Table 3 (Ministry of Agriculture and Forestry, 2022).

**Table 3:** Number of farmers registered in the Farmer Registration System in Malkara district of Tekirdağ province.

Production year	Number of farmers (person)
2017	6364
2018	6348
2019	6201
2020	6142
2021	6262

The products with the highest cultivation rate in in open field agriculture, which is mainly carried out in Malkara district, are cereals. Wheat is one of the most important cereals cultivated in the district. Since wheat is a strategic plant that has been cultivated for years in region conditions, its cultivation increases every year. Wheat, which is the raw material of flour, has wide cultivation areas in the region. Modern agricultural practices in the district, use of certified pure seeds, timely and adequate fertilization, effective struggle against diseases and pests, and climate have contributed greatly to the increase in yield in wheat cultivation areas. This increase in yield has enabled the increase in wheat production in the district every year. The information about the wheat cultivation area,

yield and production amount for the last five years in Malkara district is shown in Table 4.

**Table 4:** Data of wheat cultivation area, yield and production amount in Malkara district of Tekirdağ province.

Production year	Cultivation area(da)	Yield (kg/da)	Production amount (ton)
2017	373.150	450	168.098
2018	369.542	320	115.483
2019	367.388	464	170.372
2020	373.463	380	141.720
2021	367.893	527	194.042

Although there are small fluctuations in the cultivation area seen in Table 4 depending on the years, it has been seen that the production amount has increased with the modern agricultural methods applied in the district.

After wheat, the most cultivated plant in the district is sunflower. Sunflower, an oily plant, is a plant that has been cultivated in the region for years and contributes to the local economy. The increase in the demand for oil crops in our country has led to an increase in the agricultural areas where sunflowers are cultivated in recent years. The cultivation of sunflower in the region has been gaining momentum in recent years and has contributed positively to the amount of yield obtained from the unit area. The information on the cultivation area, yield and production amount of the sunflower plant in the last five years of Malkara district is shown in Table 5 (TURKSTAT, 2022).

**Table 5:** Data of sunflower cultivation area, yield and production amount in Malkara district of Tekirdağ province.

Production year	Cultivation area(da)	Yield (kg/da)	Production amount (ton)
2017	287.350	225	64.555
2018	292.364	241	70.320
2019	257.128	250	64.394
2020	281.844	246	69.325
2021	329.650	240	78.955

As seen in Table 5, although there are small fluctuations in the amount of yield depending on the years, the increase in the amount of cultivation area has made a positive contribution to the increase in the total production amount.

Livestock farming is predominantly carried out besides agriculture in Malkara district. The cultivation of forage crops is of great importance in the district due to the widespread livestock farming. Forage crops in the district are the most cultivated crops after sunflower. There is diversity in the cultivated forage plants. The most cultivated forage crops in the region can be listed as barley (forage), corn (silage), alfalfa (green grass), Hungarian vetch (green grass).

The information on the cultivation area, yield and production amount of the forage crops in the last five years of Malkara district is shown in Table 6, Table 7, Table 8 and Table 9 (TURKSTAT, 2022).

**Table 6:** Data of barley (forage) cultivation area, yield and production amount in Malkara district of Tekirdağ province.

Production year	Cultivation area(da)	Yield (kg/da)	Production amount (ton)
2017	32.034	554	17.748
2018	33.542	319	10.694
2019	35.000	505	17.670
2020	23.030	427	9.845
2021	26.700	576	15.373

**Table 7:** Data of corn (silage) cultivation area, yield and production amount in Malkara district of Tekirdağ province.

Production year	Cultivation area(da)	Yield (kg/da)	Production amount (ton)
2017	22.900	4640	106.250
2018	24.140	4638	111.962
2019	25.000	4636	115.900
2020	19.000	4595	87.300
2021	17.000	4894	83.200

**Table 8:** Data of alfalfa (green grass) cultivation area, yield and production amount in Malkara district of Tekirdağ province.

Production year	Cultivation area(da)	Yield (kg/da)	Production amount (ton)
2017	11.675	4400	51.370
2018	12.800	4600	58.880
2019	13.500	4600	62.100
2020	12.000	4600	55.200
2021	10.200	4600	46.920

**Table 9:** Data of Hungarian vetch (green grass) cultivation area, yield and production amount in Malkara district of Tekirdağ province.

Production year	Cultivation area(da)	Yield (kg/da)	Production amount (ton)
2017	11.000	2200	24.200
2018	12.000	3000	36.000
2019	10.000	3000	30.000
2020	9.000	3000	27.000
2021	8.250	3000	24.750

Canola is the most cultivated plant after forage crops in Malkara district. Canola plant is cultivated in the region as an alternative product for oil crops. It is seen that the cultivation areas of the canola plant, which is widely cultivated in the Thrace region, have been on the rise in recent years. The increase in cultivation areas, affiliated with the applied modern agricultural methods, naturally resulted in an increase in yield and total production amount. The canola cultivation areas, yield and production amount for the last five years of Malkara district are shown in Table 10 (TURKSTAT, 2022).

**Table 10:** Data of canola cultivation area, yield and production amount in Malkara district of Tekirdağ province.

Production year	Cultivation area(da)	Yield (kg/da)	Production amount (ton)
2017	4.000	271	1.083
2018	8.985	330	2.965
2019	15.530	360	5.591
2020	13.000	360	4.680
2021	18.000	389	7.010

An image taken from a sunflower field in Malkara district of Tekirdağ province is presented in Figure 3, and another image taken from a canola field is presented in Figure 4 below.



Figure 3. A view from the sunflower field of Kozyörük neighborhood of Malkara district.



**Figure 4:** View of canola field from Balabancık neighborhood of Malkara district.

It is seen that the yield amounts of the plants cultivated in the district are on the rise every passing year. Correct and timely fertilization comes at

the beginning of this increase in yield. In the district where livestock farming is intense, well-burned farm manure obtained from animal feces is used in the agricultural areas. Chemical fertilizers, which are widely used in the district, and solid organomineral fertilizers, which have recently been widely used, contribute greatly to this yield increase. The use of chemical fertilizers for the last five years and solid organomineral fertilizers for the last three years are shown in Table 11 and Table 12 (Ministry of Agriculture and Forestry, 2022).

**Table 11:** Chemical fertilizer amounts used in Malkara district of Tekirdağ province.

Production year	Chemical fertilizer amount (kg)
2017	39.215,725
2018	28.440,000
2019	38.165,550
2020	38.052,000
2021	27.405,610

**Table 12:** Solid organomineral fertilizer amounts used Malkara district of Tekirdağ province.

Production year	Number of user	Total amount of use (kg)
2019	181	592.250
2020	171	459.475
2021	565	1.634.360

When the tables are examined, a serious decrease has been observed in the use of chemical fertilizers in recent years. The main reason for this decrease is the increase in fertilizer prices. In recent years, with the Solid Organic-Organomineral Fertilizer Support, which has an important support from the Ministry of Agriculture and Forestry in our country, the use of

organic fertilizers by the farmers in our district has started to become widespread.

These fertilizers can be easily obtained by the farmers from 24 fertilizer dealers in the district. Besides, there are 16 Plant Protection Products Dealers providing easy access to plant protection products used in the struggle against plant diseases. The rate of mechanization is high in the region and there are 10 Agricultural Equipment and Machinery Production-Sales enterprises in the district.

## **2.2. Animal Production**

Malkara district has an important place in Turkey in terms of animal presence and quality. In general, cattle and small cattle breeding is widespread. Although not in large quantities, beekeeping and egg poultry farming are carried out. Dairy farming is done intensively in the district. The majority of the cows are pure Holstein and Simmental breeding with high productivity. Besides, it is an open market in terms of breeding animals.

There are two feed factories in the district. Producers can meet their animal feed needs from this factory, as well as easily obtain them from feed factories, producer cooperatives and grain producers in the surrounding provinces and districts. Besides, by means of the various forage plants cultivated in the district, producers can produce their own feed. There is one Meat Combination Plant belonging to Tekirdağ Metropolitan Municipality in the district. Thus, the slaughter of the animals is easily done under veterinary control in a hygienic environment in accordance with Islamic conditions. Private veterinary clinics serving producers are considerably common. Besides, vaccination and tagging services are provided to the animals by the District Directorate of Agriculture and Forestry.

Various supports are provided to the producers by the Ministry of Agriculture and Forestry. Besides, long-term credits are provided by public

and private banks with low interest rates. Since all these positive developments have contributed positively to livestock activities, the number of animals in the district has started to increase. Data of cattle and sheep and goat existence in Malkara district are shown in Table 13 (TUIK, 2022).

**Table 13:** Animal existence in Malkara district of Tekirdağ province.

Production year	Sheep and goat existence	Cattle existence
2017	58.490	55.136
2018	58.550	55.021
2019	59.360	54.317
2020	60.250	55.845
2021	66.400	56.377

### 2.3. Agricultural Economics and Subsistence

A large part of the economy of Malkara district is based on agriculture and livestock farming. The size and productivity of the agricultural lands in the district, the adequate climatic conditions, the variety of products, the second crop cultivation after the harvest of some products, and the good irrigation opportunities are of great importance in the economic development of agriculture.

Agricultural Organization has an important place in the district. In this context, 71 Cooperatives and Unions, including 57 Agricultural Development Cooperatives, 8 Irrigation Cooperatives, 1 Aquaculture Cooperative, Dairy Producers Union, Red Meat Producers Union, Breeding Sheep-Goat Producers Union, Cattle Producers Union and Honey Producers Union, are present. There are also many local dealers who enable the farmers to market their products. Various long-term and low-interest credits are provided to the farmers by the bank with repayment at harvest time.

Most of the people living in rural areas are engaged in livestock farming as well as agriculture. The production of 'Malkara Old Kashar

Cheese' which is the geographically indicated product of the district and other cheese varieties, are intense (Anonymous, 2022e). Since the presence of large dairy factories and many small and medium-sized dairy farms in the region increases the demand for milk, milk is both easy to market and has a high economic value. Besides, support is given by the ministry for milk and meat production in the district.

Agriculture and livestock farming supports are given prevalently in the district by the Ministry of Agriculture and Forestry. Supports for plant production can be listed as Diesel and Fertilizer Support, Certified Seed Use Support, Forage Crops Support, Cereals, Legumes and Grain Corn Deficiency Payments, Oil Seeds Deficiency Payments, Solid Organic-Organomineral Fertilizer Support, Licensed Warehousing Supports. The supports given for animal production are Calf Support, Mother Sheep and Goat Support, Herd Growth and Renewal Support, Beehive Support, Herd Manager (shepherd) Employment Support, Raw Milk Support. Besides, grant supports are provided by the ministry at certain rates at certain times. The income status of people engaged in agriculture and livestock farming is generally high.

Tourism opportunities in the district are limited. Malkara Organized Industrial Zone, which was established in the district, provided job opportunities to many people in the region.

A view of Malkara Old Kashar Cheese, a geographically indicated product of Malkara district, is given in Figure 5 (Anonymous 2022f).



**Figure 5:** A view of geographically indicated Malkara Old Kashar Cheese (Anonymous, 2022f).

## 2.4. Population and Number of Villages

Agriculture is done intensively in Malkara district. In the post-Republican period, the majority of the population lived in rural areas, as a large part of the population was engaged in agriculture. However, in recent years, there have been migration movements from rural areas to cities with the increase in the attraction to district life. The reasons such as the restricted opportunities in rural areas, the inadequacy of social activities and the lack of interest of the young population in agriculture have caused this migration to increase. A large part of the population lives in the district center and they generally reside in the village for a certain period of time during planting and harvesting. When the production season is over, they continue to live in the district center. Generally, people with higher average age reside in the villages. Malkara is the second district with the highest number of villages in Tekirdağ. There are 77 neighborhoods in the district. 4 of these neighborhoods are central neighborhoods and 73 of them are mentioned as villages. Population data of the Malkara district for the years 1940-2020 are shown in Table 14 (Anonymous, 2022g).

**Table 14:** Population data of Malkara district of Tekirdağ province.

Year	Total population	City population	Rural population
1940	38.113	4.576	33.537
1950	44.363	5.971	38.392
1960	53.868	9.364	44.504
1970	57.720	13.108	44.612
1980	58.829	15.425	43.404
1990	62.524	20.180	42.344
2000	59.125	24.898	34.227
2010	54.315	27.787	26.528
2020	52.101	30.263	21.838

### **3. Conclusion and Suggestions**

Malkara district of Tekirdag province, whose agricultural and animal production information has been given above and evaluated according to scientific criteria, constitutes one of the important agricultural centers of our country. However, in recent years, some problems have occurred in agricultural production due to some reasons such as excessive price changes in agricultural inputs and the global climate. Moreover, these problems are present throughout our country. However, the precautions taken by the Ministry of Agriculture and Forestry and the incentives given to the producers reduce the impact of these problems and partially relieve the producers. However, the fact that the producers give more importance to soil analysis, make fertilization programs and spraying applications more professionally will positively affect the course of agricultural production. In recent years, some problems have occurred in livestock farming in the district due to the increase in feed and energy prices, and this situation needs to be solved urgently. This is a global problem and partially affects the district negatively.

The agricultural lands of Malkara district, where wheat, sunflower, silage corn, barley, canola and alfalfa are cultivated intensively, must be analyzed from year to year. Moreover, there are soil analysis laboratories authorized by the Ministry of Agriculture and Forestry in the region and at many points close to the region. Training and demonstration studies should be carried out and followed up on the use of this advantage by the producers. It is known that chemical fertilizers are used above the average of our country in Malkara district, where cereals production is intense. In this regard, it is very important to prevent the use of excessive fertilizers by following the producers and soil analyzes in terms of both reducing input

costs and preventing environmental pollution. Although the producers prefer chemical fertilizers as fertilizers, they should also be supported and informed about using organic (for example, compost, vermicompost, green manure, algae fertilizer) and organo mineral fertilizers. In this regard, the financial supports of the Ministry of Agriculture and Forestry are still continuing.

Incentive, project and demonstration studies should be carried out on the use of animal wastes as compost in the facilities where livestock farming is performed in the region and their use as organic fertilizers in agricultural areas. Although this subject is popular all over the world, it is also essential for Malkara district, considering the excessively increasing prices of chemical fertilizers today. The use of the wastes as organic fertilizers is extremely important in terms of its contribution to agricultural areas, preventing environmental pollution and also reducing agricultural inputs.

As a result, agriculture and livestock farming are important means of existence in Malkara district, and it is extremely important to increase incentives and support and training programs in this regard. Malkara district is one of the important centers of agriculture against the increasing population of the country. It is seen as an obligatory requirement to include agriculture among the priority issues with the help of incentives, different supports and farmer training programs to be performed in the future in the region. Considering its past and present contributions to agricultural production, it is estimated that Malkara district will add value to agricultural production in the future. In terms of geographical location and soil structure, Malkara district has the characteristics of a full agricultural city and is an important center of attraction in terms of agricultural production.

## REFERENCES

- Anonymous, 2022a. Tekirdağ Province Map. Tekirdağ Metropolitan Municipality. Access address: [https://www.tekirdag.bel.tr/content/WebSource/image/harita/idari\\_harita\\_big.jpg](https://www.tekirdag.bel.tr/content/WebSource/image/harita/idari_harita_big.jpg), Access date: 20.07.2022.
- Anonymous, 2022b. Malkara County Map. Access address: <https://www.trakyanet.com/trakya/tekirdag/malkara/malkara-koyleri.html>, Access date: 21.07.2022.
- Anonymous, (2022c). Wikipedia. Access address: <https://tr.wikipedia.org/wiki/Malkara#:~:text=%C4%B01%C3%A7ede%20y%C3%BCksek%20da%C4%9Flar%2C%20vadiler%20yoktur,%2C%20Tekirda%C4%9F%2DGelibolu%20istikametinde%20uzan%C4%B1rlar>. Access date: 19.07.2022.
- Anonymous, (2022d). Turkish State Meteorological Service. Access address: <https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?m=TEKIRDAG> Access date: 26.07.2022
- Anonymous, (2022e). Turkish Patent and Trademark İnstitutiob. Access address: <https://ci.turkpatent.gov.tr/Files/GeographicalSigns/8ffe5d9b-9204-43e8-b397-9cee10abf245.pdf> Access date: 05.08.2022.
- Anonymous, (2022f). Malkara Old Kashar Cheese. Access address: <https://www.habernediyor.com/tarim/cografisi-isaretli-malkara-eski-kasar-peyniri-h22087.html> Access date: 16.08.2022
- Anonymous, (2022g). Wikipedia. Access address: <https://tr.wikipedia.org/wiki/Malkara#N%C3%BCfus> Access date: 21.07.2022.
- Bellitürk, K., 2011. Edirne İli Uzunköprü İlçesi Tarım Topraklarının Beslenme Durumlarının Belirlenmesi. Tekirdağ Ziraat Fakültesi Dergisi (JOTAF), Tekirdağ, 8 (3): 8-15.
- Bellitürk, K., 2019. Changes in Plant Nutrition for a Sustainable Agriculture: Past, Present and Future. ISPEC International Conference on Agriculture and Rural Development-III, 20-22 December 2019, 590-600, Van-Turkey.
- Bellitürk, K. and Çelik, A., 2022. Some Assessments on the Fertilizer Need of Winter Wheat (*Triticum aestivum* L.) Widely Grown in the Thrace Region. International Conference on Global Practice of Multidisciplinary Scientific Studies, pp. 976-985, 6-8 March, Cyprus.
- Republic of Türkiye Ministry of Agriculture and Forestry, (2022).
- TURKSTAT, (2022). Turkish Statistical Institute. Access address: <https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr> Access date: 21.07.2022.

## CHAPTER 5

### EFFECTIVE USE OF BIOCHAR AMENDMENT TO ALLEVIATE DROUGHT AND SALINITY STRESS IN THE PRODUCTION OF LEGUMES AND VEGETABLES

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## 1. INTRODUCTION

The increase in temperatures of last decade and alarming projection in coming decades with increase in CO<sub>2</sub> and more variable precipitations due to climate changes are hampering agriculture production. Soon some plant species will not be able to rely on their own defense and adaptation mechanisms (Hatfield and Walthall, 2014). Legumes and vegetables are among vulnerable species in which yield losses are being observed. Effects of climate changes particularly abiotic stresses are detrimental to grain quality and composition of food legumes (Sarkar et al., 2021). Moreover, abiotic stresses such as heat, cold, drought, and salinity are known to considerably reduce vegetables yields (Malhi et al., 2021; Shimira and Taşkın, 2022). Legumes and vegetables provide innumerable dietary and health benefits. Because of their higher protein contents, fibers, essential amino acids, minerals, vitamins, and phytochemicals, these crops are gaining popularity among consumers and the food industry. Legumes and vegetables, which contain all of the aforementioned phytonutrients, may safeguard humans from chronic diseases while also addressing pressing global nutrition issues (Dias, 2012; Keskin et al, 2022).

Nowadays, the only promising solution to mitigate these challenges is to rely on sustainable agriculture that involves the use of resilient crop species with high yield and limited environmental impact (Tian et al, 2020). Commonly, sustainable agriculture is defined as crop production that does not impact the environment, biodiversity, and agricultural crop quality. Producing crops sustainably optimizes the system's ability to maintain constant quality and yield over time without increasing the necessity for agricultural chemical inputs to ensure continued management (Imadi et al., 2016). Sustainable crop production involves adding organic matter to the soil, improving nutrient quality, adopting integrated pest management, and reducing pesticide use.

Most of these environmentally friendly practices are aimed at preserving biodiversity and ensuring food security, as well as lowering global carbon footprint and greenhouse gas emissions (Imadi et al., 2016; Shimira et al., 2021).

Additionally, breakthroughs in plant breeding technologies have been employed by scientists and breeders for crop quality and yield improvement through the development of improved pest-resistant and multi-stress tolerant crop varieties. All of these research initiatives have helped to limit yield loss due to climate adversities, weeds, diseases, and insects while also greatly increasing yields. For the future, novel technologies such as precision farming, which uses GPS, sensors, and drones are expected to straighten sustainable agricultural practices (Smyth et al., 2022). In the sake of reducing climate adversities such as abiotic stresses biochar application is greatly considered by scientific community as an environmentally friendly solution for soil fertility replenishment, contaminant immobilization, in situ carbon sequestration, and wastewater treatment. Thus, biochar is made through the partial or complete combustion of biomass or organic waste in absence of oxygen. In other words, it is made under the controlled thermochemical combustion of organic materials (Ali et al., 2017; Egamberdieva et al., 2022; Wang et al., 2022). Although in these days, biochar material is re-gaining popularity, anthropological reports shows that it was used in ancient time by the pre-Columbian Indios as a soil supplement. It was regularly applied to soils to preserve fertility which have given a nutrient-rich soils known as Terra Preta do Indio (Nguyen et al., 2022).

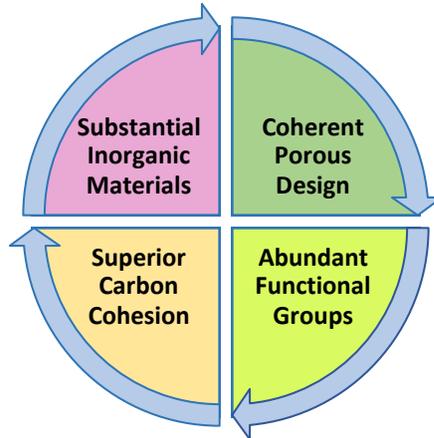
In this chapter, we highlighted all different research findings on the role of biochar application in mitigating abiotic stresses particularly drought and soil salinity that are responsible of huge crop yield losses. Prospective use of biochar for soil health improvement and its role in rising yield of legumes and

vegetables as well as its mechanisms of actions that allows crop development under drought and saline stress conditions were also discussed in this chapter.

## **2. BIOCHAR AMENDMENT AND ITS MECHANISMS OF ACTIONS**

Biochar is now widely used for soil improvement in agricultural production systems and environmental remediation, owing to its physico-chemical properties (Figure 1). These features improve the overall biological and physio-biochemical status of the soil. It is also one of the key reasons why biochar amendment is becoming increasingly popular (Egamberdieva et al., 2022; Imran et al., 2022). Biochar is a carbon-rich material with key attributes including high alkalinity and high surface adsorption potential. Moreover, biochar is a soil conditioner, a bio-stimulant and a fertilizer that boosts crop production. Biochar is also frequently combined with other materials to create biochar composites that can be used as soil amendments. Biochar amendment has been proposed as a means of combating soil salinization and drought. Salinization degrades soil biochemical and physical properties, particularly carbon availability and microbial activity, decreasing soil productivity or even rendering it unproductive once salt levels surpass acceptable limits (Lee et al., 2022; Nguyen et al., 2022). While, Drought stress reduces soil water availability, resulting in insufficient water for plant growth (Giordano et al. 2021). This sustainable approach could enhance productivity while also addressing the food security crisis, which is exacerbated by global warming (Lee et al., 2022). Biochar application has been shown to directly alter soil structure and chemical fertility, resulting in increased crop productivity. Biochar composites are densely enriched with immobilization/degradation components that ensure long-term remediation of polluted soils with minimal life cycle impact (Imran et al., 2022; Wang et al., 2022). Additionally, it is stated that specific biochar combinations stimulate the overall nitrogen status

of the soil under saline conditions (Moradi et al., 2019). It is also reported that biochar amendment can reverse the drought adverse impacts on soils and plants (Gavili et al., 2019).



**Figure 1:** Physicochemical properties of biochar.

Biochar is made through pyrolysis and depending on the heating rate, temperature, residence time, and pressure, pyrolysis can be classified as rapid or slow. Additionally, several types of biochar can be created based on the intended purpose (Yaashikaa et al., 2020). Rotating kilns, gasification, vertical silo-type reactors, and hydrothermal carbonization are all common ways for producing biochar. Depending on pyrolysis settings such as temperature, aeration, time, speed, high electrical conductivity (EC), high pH, high mineral content (Na, K, Fe, Mg, etc.), ash concentrations, and low volatile matter, it is estimated that total organic carbon accounts for about 30 to 70% of biochar (Ali et al., 2017). These types of pyrolysis and the type of raw materials employed (feedstock), are just some of the many factors that might affect biochar quality (Joseph et al., 2021; Lefebvre et al., 2020). Slow pyrolysis, for instance, is carried out under limited oxygen (O<sub>2</sub>) settings, in which agriculture waste is burned in a furnace for two hours until the appropriate

temperature (300-700 °C) is reached, with an average heating speed of 5 °C min<sup>-1</sup> (Saleem et al., 2022).

### **3. BIOCHAR APPLICATION AND ITS EFFECTS IN ALLEVIATING SOIL SALINITY STRESS**

Salinization is one of the most common soil degradations in farmland, especially in arid and semiarid areas. Soil salinization is exacerbated by dry climate, excessive evaporation, and irrigation-based agriculture, which introduces soluble salts including Na<sup>+</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and HCO<sub>3</sub><sup>-</sup> into the soil (Lee et al., 2022). High salinization levels can lead to the loss of evolving soil resources, affecting agricultural production and soil health. Soil salinization refers to saline, sodic, and alkaline soils, which are defined as having a high salt concentration, a high sodium cation (Na<sup>+</sup>) concentration, and a high pH in the soil, often due to a high CO<sub>3</sub><sup>2-</sup> content (Daliakopoulos et al., 2016). Furthermore, Salinity alters soil biodiversity and microbial activity, resulting in lower rates of microbial respiration and carbon recycling (Yu et al., 2019). Salinity prevents water from penetrating into the soil, resulting in erosion and surface runoff. It also restricts agricultural development and ecological functions by reducing the utilization of available soil resources (Rengasamy, 2006; Gorji et al., 2020).

#### **3.1. Biochar-based mitigation of soil salinity under legumes cultivation**

To combat the adverse effects of salt stress, biochar treatment has been applied to a wide range of legumes. Various researchers have carried out field, greenhouse, and pot experiments, with encouraging outcomes. Farhangi-Abriz and Torabian (2018a) have tested biochar (10, and 20%) treatment to alleviate the negative impacts of salt stress in common bean seedlings. They observed that biochar amendment reduces the levels some endogenous stress

hormones like polyamines, polyamine oxidase, jasmonic acid, abscisic acid, and 1-aminocyclopropane-1-carboxylic acid, as well as the Na concentration. In contrast, biochar treatment improved indole-3-acetic acid levels resulting in better growth of shoot and roots of common beans. Similarly, Farhangi-Abriz and Torabian (2018b) and Karabay et al. (2021) conducted distinct biochar amendment experiments in common beans to evaluate its impact on plant growth under salinity stress conditions. Due to its unique physicochemical properties, particularly its high Na<sup>+</sup> adsorption capacity, biochar application was unanimously confirmed to be a promising strategy for increasing common bean growth in saline soils. Biochar treatment improves plant growth and biomass.

Other advantages of biochar treatment include increased chlorophyll content (a, b, and total) and key nutrient uptake (like potassium, calcium, and magnesium). It also limits Na content and uptakes. Zhang et al. (2020) also assessed the effect of biochar amendment on soybean productivity under salinity and drought stress. Soybean grain yield was considerably enhanced by biochar amendment. It also mitigates the detrimental effects of both stresses on soybean productivity and water use efficiency. Table 1 lists additional relevant findings on the effect of biochar treatment on salt-stressed legumes.

### **3.2. Biochar-based mitigation of soil salinity under vegetables cultivation**

Biochar application have been proven to be beneficial to broad variety of vegetables grown under salinity stress conditions. Several field, greenhouse and pot experiments have been conducted by various researchers and promising results were found. For instance, Hammer et al. (2015) used lettuce to investigate combined effects of biochar and arbuscular mycorrhizal fungi on its growth and physiology during salinity stress. The combined amendment

positively increased lettuce yield. Thus, it raised nutrient uptake of key elements such as phosphorus and manganese as well as improved Na/K ratio in stressed-lettuce.

**Table 1.** Key experiments on the use of biochar in legumes as salinity-mitigating treatments.

Nº	Type of Amendment	Crops	Findings	References
1.	Biochar	Mung bean	Biochar application improved plant growth (the shoot/root ratio, total root area and specific root length) and numerous parameters in mung bean such as xylem structure and plant growth regulator particularly IAA. It also enhanced relative water content. Additional, biochar amendment lowered keys stress hormones (ABA and ACC), known to promote plant senescence which result in high salt-tolerance of mung.	(Nikpour-Rashidabad et al., 2019)
2.	Biochar and CaCl <sub>2</sub>	Cowpea	Biochar and osmopriming by CaCl <sub>2</sub> were tested on cowpea seeds and examined their effects on seed germination under salinity stress. They reported that under salt stress, the combination of osmopriming and biochar application accelerated and synchronized seedling emergence and also improved seedling development, total antioxidant activity, α-amylase activity as well as the Na accumulation. The combined treatment promoted chlorophyll synthesis, sugar accumulation which lowered the oxidative damage and Na <sup>+</sup> toxicity.	(Farooq et al., 2020).

3.	Biochar	Quinoa	Biochar amendment assisted to reduce adverse effect of salt stress in quinoa crops. In addition, biochar treatment combined with an alternate root-zone drying irrigation regime boosted plant height, shoot biomass, and grain production considerably under salinity stress conditions.	(Yang et al., 2020)
4.	Biochar	Licorice	Biochar treatment helped to substantially increase shoot biomass and moderately increased root biomass. Moreover, K and N concentrations in plant tissue increased as well. The application of biochar to the soil improved enzymatic activities (proteases, acid phosphomonoesterases, hydrolase, and diacetate), as well as overall nutrient uptake.	(Egamberdieva et al., 2021)
5.	Biochar (5% and 10%)	Common bean	Biochar application diminished enzymatic activities (CAT, SOD, and POD), proline, and malondialdehyde (MDA) contents, resulting in improved plant growth due to decreased oxidative and osmotic stresses. A low dose of biochar (5%) increased plant height and leaf area, as well as leaf relative water content and chlorophyll-a concentration. However, the highest increase rates in shoot and root biomass, as well as the number of leaves, were observed with a 10% biochar application.	(Kul et al., 2021b).

Similarly, Malik et al. (2022) assessed the impact of biochar (5-7%) on lettuce growth efficiency under salinity and drought stress conditions. They

reported that small sized (< 1 mm) biochar (7%) mixed with manure significantly improved root biomass under salinity stress conditions and promoted phosphorus cycling in soil. Phosphorus use efficiency of lettuce leaves is one of the parameters that increased after the application of small particle-sized co-composted biochar. Other relevant findings on the effect of biochar treatment on salt-stressed vegetables are listed in Table 2.

**Table 2.** Key experiments on the use of biochar in vegetables as salinity-mitigating treatments.

Nº	Type of Amendment	Crops	Findings	References
1.	Biochar (5-10%)	Okra	The biochar application considerably increased salt-tolerance in okra crops. Thus, okra salt threshold was increased to more than 80% particularly with 10% dose biochar. They also observed a better growth and high yield in okra as well as high water content in the soil. Furthermore, soil bulk density was lowered after the amendment.	(Elshaikh et al., 2018)
2.	Biochar	Eggplant	Biochar treatment result in better shoot and root growth as well as in high and fruit yield of eggplant. Moreover, parameters such as stomatal conductance and photosynthetic rate were boosted. They also observed a decrease of leaf temperature and electrolyte leakage in eggplant leaf tissues.	(Parkash and Singh, 2020)
3.	Sulfur-enhanced biochar (5%) with microorganisms	Pepper	Biochar composite application resulted in amelioration of nutritional status, photosynthetic efficiency, and dehydration tolerance. They also observed significant pepper growth and	(Abd El-Mageed et al., 2020).

			yield as well as macro- and micronutrient concentrations. Other improved parameters include irrigation usage efficiency and dehydration tolerance.	
4.	Biochar % (B2), 4% (B4), and 6% (B6) for pot experiments	Licorice	Biochar amendment was favorable to the licorise growth and root system adaptation as well as nutrient uptake. Thus, the root architecture, soil enzyme activity (proteases, fluorescein diacetate hydrolase, and acid phosphomonoesterases) and nodule formation was enhanced after biochar treatment. Only, high dose biochar (6%) did not show any positive result.	(Egamberdieva et al., 2021).
5.	Biochar (5%, 10%)	Tomato	Biochar treatment significantly improved plant biomass under saline conditions. Additionally, biochar application at both doses considerably lowered the catalase, superoxide dismutase, and peroxidase activities. It also lowered malondialdehyde and proline levels, resulting in enhanced plant growth as oxidative and osmotic stresses were minimized.	(Kul et al., 2021a).
6.	Biochar and soil fungi (Trichoderma harzianum)	Spinach	The combined application of biochar and soil fungi was favorable to lower key parameters such as reactive oxygen species, membrane leakage, malondialdehyde, electrolyte leakage, and salt content in spinach under salt stress. They also reported that antioxidant upregulation was improved.	(Sofy et al., 2021)

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7.	Nanoparticles enriched biochar	Radish	The application of nanoparticles enriched biochar help to reduce malondialdehyde (MDA) and hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> ) in the early seedling growth stage of radish.	(Taqdees et al., 2022)
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Furthermore, the effect of biochar (2%, 4%, and 8%) application was evaluated on tomato growth under saline water irrigation regimes by She et al. (2018). Findings show that the addition of biochar to the soil improved vegetative growth, yield, and quality indicators. It also improved salt tolerance of tomato crops. In the soil, biochar treatment reduced transient sodium ions through adsorption and liberated mineral nutrients including calcium, potassium, and magnesium into the soil solution. Kul et al. (2021a) examined thoroughly the effect of biochar treatment on the physiology, growth and antioxidant activity of common bean grown under salt stress conditions.

Biochar treatments reduced the adverse effects of salt stress on the physiological and plant growth characteristics of common beans, according to the findings. They also detected a decrease in antioxidant activity, MDA, and H<sub>2</sub>O<sub>2</sub>, as well as a rise in relative leaf water content and total chlorophyll.

#### **4. BIOCHAR APPLICATION AND ITS EFFECTS IN ALLEVIATING DROUGHT STRESS**

Drought stress is known to limit crop production worldwide, and with ongoing climate change it may become more severe in the future. Due to this constraint, plants are experiencing growth and water use disturbance. Although plants possess their own mechanisms of defense to counter water deficit stress, which involve a variety of physiological and biochemical responses at cellular and whole organism levels, if stress persists, drought effects might be detrimental to growth process (Farooq et al, 2012). Moreover,

drought stress has also been identified as a major constraint to legume and vegetable growth and yield decline (Egamberdieva et al., 2020; Malhi et al., 2021).

It has been reported that applying biochar to crops can help lessen the negative effects of drought (Ali and Elshaikh, 2022). Because of its negatively charged surfaces and large surface area, biochar is recommended in drought mitigation strategies because it improves soil water retention capacity. Other advantages of using biochar include the reduction of soil bulk density and the absorption of nutrients that are then gradually released to the plants (Hafeez et al., 2017). Additionally, the use of biochar creates favorable conditions for microbial proliferation and survival (Egamberdieva et al., 2020). For instance, drought-stressed crops benefit from biochar amendment in terms of physiological, morphological, performance, and nutrient uptake. Water retention, as well as the physical and biological qualities of soils, have also been reported to improve. In biochar-amended soil, sodium ( $\text{Na}^+$ ) ion uptake was reduced, resulting in increased uptake of other cations such as potassium ( $\text{K}^+$ ) by plants (Ali and Elshaikh, 2022).

#### **4.1. Biochar-based mitigation of drought stress in legumes cultivation**

The effect of biochar application on important legumes grown under water deficiency stress has been investigated in a variety of field and pot experiments. It is also been observed that biochar treatment improves physiological qualities and legume growth by increasing root and shoot dry mass, as well as yield through a large number of pods in major legumes including common bean (*Phaseolus vulgaris* L.), garden pea (*Pisum sativum* L.), and others (Egamberdieva et al., 2020). Table 3 summarizes the other important findings on the effect of biochar application on drought-stressed legumes.

**Table 3.** Key experiments on the use of biochar in legumes as drought-mitigating treatments.

Nº	Type of Amendment	Crops	Findings	References
1.	Biochar	Lentil	The use of biochar (5 t ha <sup>-1</sup> ) was proven to be beneficial to soil quality and plant growth. Biochar doses of 5 to 15 t ha <sup>-1</sup> were found to be unfavorable to lentil growth and soil quality. Weed development was also exacerbated by these high doses.	(Safaei et al., 2018).
2.	Biochar (1.25, 2.5 and 5%)	soybean	Biochar amendment raised the leaf area, stomatal conductance and the greenness index of soybean leaves. Furthermore, the biochar application at 1.25% dose was found favorable for raising pod and plant height as well as other parameters such as water use efficiency.	(Gavili et al., 2019).
3.	Biochar with alternate root-zone drying irrigation (ARD)	Quinoa	Biochar application was proven to alleviate the combined effect of drought and salinity stress in quinoa crops. They observed an ameliorated shoot biomass, plant height, and grain yield. Furthermore, the interactive effect of biochar and ARD efficiently adjusted the balance between chemical signal (leaf ABA) and hydraulic signal (leaf water potential).	(Yang et al., 2020).

4.	Biochar and CaCl <sub>2</sub>	Cowpea	Biochar and osmopriming by CaCl <sub>2</sub> were essayed on cowpea seeds and evaluated their impact on seed germination under drought stress. It was found that It boosted cowpea effectiveness under water deficit conditions by synchronizing seedling emergence and promoting growth, as well as CO <sub>2</sub> assimilation, chlorophyll synthesis, nutrient uptake with minimal oxidative damage, and osmolyte accumulation.	(Farooq et al., 2021).
5.	Biochar	Soybean	High yield was obtained with biochar application at 100 t ha <sup>-1</sup> . It also significantly increased crop growth rate and total biomass production of soybean under drought stress condition in a clay soil. Stress tolerance in soybean was enhanced by biochar application and key nutrient like potassium was made available to soybean.	(Mannan et al., 2021).
6.	Biochar	Soybean	Chlorophyll content and the soybean growth was ameliorated after biochar application. Biochar reversed the effect of drought stress by modulating the plant physiology and biochemistry whereby it lowered proline, abscisic acid and sucrose contents. Malondialdehyde and hydrogen peroxide are	(Gullap et al, 2022).

			other parameter reduced by biochar amendment.
7.	Biochar and Plant growth promoting rhizobacteria (PGPR)	Canola	The combination of biochar and PGPR ameliorated epidermal vigor and stomatal physiology in canola ( <i>Brassica napus</i> L.). hydrogen peroxide, malondialdehyde, and osmolyte content such as proline are among parameters lowered after the combined amendment.
			(Lalay et al., 2022).

For instance, Mannan et al (2016) revealed that applying biochar at a high dose (50 t ha<sup>-1</sup>) boosted soybean (*Glycine max*. L.) yields and improved overall drought tolerance. As a result, the number of pods produced was higher than in untreated soybean crops. They also noticed high accumulation of water and proline in the leaves, as well as less chlorophyll degradation. Crop height, relative water content, and chlorophyll content are among the other variables that have increased. Likewise, Hafeez et al. (2017) demonstrated that a biochar amendment of at least 20 t ha<sup>-1</sup> can assist soybean (*Glycine max* (L.) Merr.) seedlings cope with water stress during germination and seedling growth. Biochar application reduced proline and sugar levels while also improving a number of important indices such as seed vigor, shoot length, membrane stability index, carotenoid, and chlorophyll content.

The broad bean or faba bean (*Vicia faba* L.), another prominent legume, was selected to assess the effect of biochar on the growth and nutritional uptake of the said beans cultivated in a sandy loam soil under drought stress. The addition of biochar (2–4%) to the soil boosted plant biomass and growth, as well as nodule formation. It also enhanced the nutritional value of the soil and nutrient uptake, particularly P and K (Egamberdieva et al., 2020).

Similarly, El-Mageed et al. (2021) examined the effect of acidified biochar amendment on faba bean (*Vicia faba* L.) growth and productivity, as well as its involvement in reducing the negative effects of salt stress. After applying acidified biochar (5–10 t ha<sup>-1</sup>), the authors reported a considerable increase in seed yield. They also emphasized its function in improving faba bean growth and physiological responses, as well as soil qualities in general.

#### **4.2. Biochar-based mitigation of drought stress in vegetables cultivation**

Biochar has been recommended by several researchers as a promising amendment to improve long-term water and nutrient status, as well as crop yield. As a result, this extremely porous pyrolyzed substance retains more water, which enables water use efficiency by crops (Mulcahy et al., 2013; Agbna et al., 2017; Yildirim et al., 2021). Research in vegetable crops showed promising results. For instance, Gavili et al. (2018) evaluated biochar effect in mitigating the adverse effects of drought in spinach, and after field experiment they found that an application of low level biochar (1.25%) can provide positive impacts on the plant's growth and yield. The application increased some physiological quality such as water use efficiency and shoot dry matter yield.

Likewise, a combined effect of biochar application with Arbuscular mycorrhizal fungi (AMF) treatment was assessed by Jabborova et al. (2021) in pot experiments on Okra (*Abelmoschus esculentus*) under drought stress. After applying biochar alone, they noticed a large increase in plant growth and root morphological traits, as well as a considerable increase in chlorophyll 'a' content and microbial biomass. While AMF alone enhanced various plant development indices such as root dry, plant shoot and height weights, as well as root diameter and volume. The combination treatment of biochar and AMF

treatment showed the maximum values of plant growth indices and root morphological traits, as expected. Furthermore, enzymatic activities, notably dehydrogenase alkaline phosphatase and fluorescein diacetate enzyme activities, increased with both combined amendments. Table 4 summarizes the other major findings in drought-stressed vegetables.

**Table 4.** Key experiments on the use of biochar in vegetables as drought-mitigating treatments.

N°	Type of Amendment	Crops	Findings	References
1.	Biochar	Tomato	Biochar soil amendment increases tomato seedling resistance to drought in sandy soils. The results demonstrate that, in sandy substrates, 30% (v/v) biochar, concentrated in seedling root zones, significantly increases seedling resistance to wilting.	(Mulcahy et al., 2013)
2.	Biochar	Spinach	Biochar amendment (25 t ha <sup>-1</sup> ) was found suitable to reduce adversity of drought stress in spinach. It significantly increased stomatal conductance, greenness index, and leaf area. Additionally, it also improved plant growth parameters.	(Gavili et al., 2016).
3.	Biochar	Tomato	Biochar has a positive impact on tomato physiology, growth, irrigation water use efficiency, fruit quality, and yield. It also enhance soil water and nutrient status by increasing total nitrogen and soil organic matter, while considerably lowering soil nitrate nitrogen and ammonium nitrogen levels.	(Agbna et al., 2017).

4.	Biochar	Tomato	Tomato seedling growth (dry weights of shoots and roots) and biomass were enhanced dramatically following biochar treatment, whereas chlorophyll content was altered. Furthermore, biochar significantly lowered plant antioxidants and enzymatic activities (superoxide dismutase and peroxidase), as well as malondialdehyde. High doses of biochar (6%) were found to be effective in improving soil physiochemical parameters as well as the physiology of tomato seedlings.	(Khan et al., 2019)
5.	Biochar (2%)	Sugar Beet	Biochar amendment (2%) resulted in the highest shoot dry matter of the sugar beet crops. Similarly, large doses (1.0–2%) of biochar enhanced nitrogen, phosphate, and potassium concentrations. In summary, biochar treatments boosted the growth and development of sugar beet crops.	(Durukan et al., 2020).
6.	Biochar (2%) and compost (2%)	Sweet pepper	The overall impact of biochar (2%) and compost (2%) considerably boosted yield, pepper growth, and water use efficiency.	(Obadi et al., 2020).
7.	Biochar	Cabbage	Biochar application enhanced the photosynthetic activity, plant growth, nutrient uptake in cabbage seedlings under water deficit conditions.	(Yildirim et al., 2021).

## **5. CONCLUSION AND PERSPECTIVE**

Biochar amendment was shown as effective in the mitigation of drought and salt stress in pot, field, and greenhouse experiments. The key evidences compiled in this chapter clearly show that different doses and forms of biochar applications are invaluable to legume and vegetable crops grown in saline and drought conditions, which can have a substantial impact like increased crop biomass and yield. When applied to the soil, biochar improves soil health and fertility, makes key nutrients more available to crops, and increases photosynthetic activity. Other advantages include increased soil nutrient retention and soil enzyme activity.

Nevertheless, the effectiveness of biochar in mitigating drought and salinity stress is dependent on the pyrolysis raw material or feedstock type, pyrolysis temperature and time, soil type and properties, and crop species. Biochar amendments are a valuable asset for sustainable agriculture, and their actions in mitigating the negative effects of some abiotic stresses have been described. This chapter sheds light on its numerous advantages, which can be maximized in the production of legumes and vegetables as well as other crops.

## REFERENCES

- Abd El-Mageed, T. A., Rady, M. M., Taha, R. S., Abd El Azeam, S., Simpson, C. R., & Semida, W. M. (2020). Effects of integrated use of residual sulfur-enhanced biochar with effective microorganisms on soil properties, plant growth and short-term productivity of Capsicum annuum under salt stress. *Scientia Horticulturae*, 261, 108930. <https://doi.org/10.1016/j.scienta.2019.108930>
- Agbna, G. H., Dongli, S., Zhipeng, L., Elshaikh, N. A., Guangcheng, S., & Timm, L. C. (2017). Effects of deficit irrigation and biochar addition on the growth, yield, and quality of tomato. *Scientia Horticulturae*, 222, 90-101. <https://doi.org/10.1016/j.scienta.2017.05.004>
- Ali, A. B., & Elshaikh, N. A. (2022). Performance of Biochar under Diminish Water Stress in Plants. *Communications in Soil Science and Plant Analysis*, 53(1), 1-16. <https://doi.org/10.1080/00103624.2021.1984508>
- Ali, S., Rizwan, M., Qayyum, M. F., Ok, Y. S., Ibrahim, M., Riaz, M., Arif, M. S., Hafeez, F., Al-Wabel, M. I., & Shahzad, A. N. (2017). Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. *Environmental Science and Pollution Research*, 24(14), 12700-12712. <https://doi.org/10.1007/s11356-017-8904-x>
- Daliakopoulos, I. N., I. K. Tsanis, A. Koutroulis, N. N. Kourgialas, A. E. Varouchakis, G. P. Karatzas, and C. J. Ritsema (2016). "The threat of soil salinity: A European scale review." *Science of the total environment* 573: 727-739. <https://doi.org/10.1016/j.scitotenv.2016.08.177>
- Dias, J. S. (2012). Nutritional quality and health benefits of vegetables: a review. *Food and Nutrition Sciences*, 3(10), 1354-1374. <https://doi.org/10.4236/fns.2012.310179>
- Durukan, H., Demirbas, A., & Turkekul, I. (2020). Effects of Biochar Rates on Yield and Nutrient Uptake of Sugar Beet Plants Grown under Drought Stress. *Communications in Soil Science and Plant Analysis*, 51(21), 2735-2745. <https://doi.org/10.1080/00103624.2020.1849257>
- Egamberdieva, D., Alaylar, B., Kistaubayeva, A., Wirth, S., & Bellingrath-Kimura, S. D. (2022). Biochar for improving soil biological properties and mitigating salt stress in plants on salt-affected soils. *Communications in Soil Science and Plant Analysis*, 53(2), 140-152. <https://doi.org/10.1080/00103624.2021.1993884>
- Egamberdieva, D., Ma, H., Alaylar, B., Zoghi, Z., Kistaubayeva, A., Wirth, S., & Bellingrath-Kimura, S. D. (2021). Biochar Amendments Improve Licorice (*Glycyrrhiza uralensis* Fisch.) Growth and Nutrient Uptake under Salt Stress. *Plants*, 10(10), 2135. <https://doi.org/10.3390/plants10102135>
- Egamberdieva, D., Zoghi, Z., Nazarov, K., Wirth, S., & Bellingrath-Kimura, S. D. (2020). Plant growth response of broad bean (*Vicia faba* L.) to biochar amendment of loamy sand soil under irrigated and drought conditions. *Environmental Sustainability*, 3(3), 319-324. <https://doi.org/10.1007/s42398-020-00116-y>

- El-Mageed, T. A. A., Belal, E. E., Rady, M. O. A., El-Mageed, S. A. A., Mansour, E., Awad, M. F., & Semida, W. M. (2021). Acidified biochar as a soil amendment to drought stressed (*Vicia faba* L.) plants: influences on growth and productivity, nutrient status, and water use efficiency. *Agronomy*, 11(7), 1290. <https://doi.org/10.3390/agronomy11071290>
- Elshaikh, N. A., Zhipeng, L., Dongli, S., & Timm, L. C. (2018). Increasing the okra salt threshold value with biochar amendments. *Journal of plant interactions*, 13(1), 51-63. <https://doi.org/10.1080/17429145.2017.1418914>
- Farhangi-Abriz, S., & Torabian, S. (2018a). Biochar increased plant growth-promoting hormones and helped to alleviate salt stress in common bean seedlings. *Journal of Plant Growth Regulation*, 37(2), 591-601. <https://doi.org/10.1007/s00344-017-9756-9>
- Farhangi-Abriz, S., & Torabian, S. (2018b). Effect of biochar on growth and ion contents of bean plant under saline condition. *Environmental Science and Pollution Research*, 25(12), 11556-11564. <https://doi.org/10.1007/s11356-018-1446-z>
- Farooq, M., Hussain, M., Wahid, A., Siddique, K.H.M. (2012). Drought Stress in Plants: An Overview. In: Aroca, R. (eds) *Plant Responses to Drought Stress*. Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-642-32653-0\\_1](https://doi.org/10.1007/978-3-642-32653-0_1)
- Farooq, M., Rehman, A., Al-Alawi, A. K., Al-Busaidi, W. M., & Lee, D. J. (2020). Integrated use of seed priming and biochar improves salt tolerance in cowpea. *Scientia Horticulturae*, 272, 109507. <https://doi.org/10.1016/j.scienta.2020.109507>
- Farooq, M., Romdhane, L., Rehman, A., Al-Alawi, A. K., Al-Busaidi, W. M., Asad, S. A., & Lee, D. J. (2021). Integration of seed priming and biochar application improves drought tolerance in cowpea. *Journal of Plant Growth Regulation*, 40(5), 1972-1980. <https://doi.org/10.1007/s00344-020-10245-7>
- Gavili, E., Moosavi, A. A., & Haghighi, A. A. K. (2019). Does biochar mitigate the adverse effects of drought on the agronomic traits and yield components of soybean?. *Industrial crops and products*, 128, 445-454. <https://doi.org/10.1016/j.indcrop.2018.11.047>
- Gavili, E., Moosavi, A. A., & Moradi Choghamarani, F. (2018). Cattle manure biochar potential for ameliorating soil physical characteristics and spinach response under drought. *Archives of Agronomy and Soil Science*, 64(12), 1714-1727. <https://doi.org/10.1080/03650340.2018.1453925>
- Gavili, E., Mousavi, S., & Kamgar, H. A. (2016). Effect of cattle manure biochar and drought stress on the growth characteristics and water use efficiency of Spinach under greenhouse conditions. *Iranian Journal of Water Research in Agriculture*, 30(2), 243-259.
- Giordano, M., Petropoulos, S. A., & Roupael, Y. (2021). Response and defense mechanisms of vegetable crops against drought, heat and salinity stress. *Agriculture*, 11(5), 463. <https://doi.org/10.3390/agriculture11050463>
- Gorji, T., Yildirim, A., Hamzehpour, N., Tanik, A., & Sertel, E. (2020). Soil salinity analysis of Urmia Lake Basin using Landsat-8 OLI and Sentinel-2A based spectral indices and electrical conductivity measurements. *Ecological Indicators*, 112, 106173. <https://doi.org/10.1016/j.ecolind.2020.106173>

- Gullap, M. K., Severoglu, S., Karabacak, T., Yazici, A., Ekinci, M., Turan, M., & Yildirim, E. (2022). Biochar derived from hazelnut shells mitigates the impact of drought stress on soybean seedlings. *New Zealand Journal of Crop and Horticultural Science*, 1-19. <https://doi.org/10.1080/01140671.2022.2079680>
- Hafeez, Y., Iqbal, S., Jabeen, K., Shahzad, S., Jahan, S., & Rasul, F. (2017). Effect of biochar application on seed germination and seedling growth of *Glycine max* (L.) Merr. Under drought stress. *Pakistan Journal of Botany*, 49(51), 7-13.
- Hammer, E. C., Forstreuter, M., Rillig, M. C., & Kohler, J. (2015). Biochar increases arbuscular mycorrhizal plant growth enhancement and ameliorates salinity stress. *Applied soil ecology*, 96, 114-121. <https://doi.org/10.1016/j.apsoil.2015.07.014>
- Hatfield, J. L., & Walthall, C. L. (2014). Climate change: Cropping system changes and adaptations. *Encyclopedia of Agriculture and Food Systems*. Elsevier, 256-65. <https://doi.org/10.1016/B978-0-444-52512-3.00003-6>
- Imadi, S.R., Shazadi, K., Gul, A., Hakeem, K.R. (2016). Sustainable Crop Production System. In: Hakeem, K., Akhtar, M., Abdullah, S. (eds) *Plant, Soil and Microbes*. Springer, Cham. [https://doi.org/10.1007/978-3-319-27455-3\\_6](https://doi.org/10.1007/978-3-319-27455-3_6)
- Imran, S., Sarker, P., Hoque, M. N., Paul, N. C., Mahamud, A., Chakroborty, J., Tahjib-Ul-Arif, Abdel Latef, A. A. H., Hasanuzzaman, M., & Solaiman, Z. (2022). Biochar actions for the mitigation of plant abiotic stress. *Crop and Pasture Science*. <https://doi.org/10.1071/CP21486>
- Jaborova, D., Annapurna, K., Al-Sadi, A. M., Alharbi, S. A., Datta, R., & Zuan, A. T. K. (2021). Biochar and Arbuscular mycorrhizal fungi mediated enhanced drought tolerance in Okra (*Abelmoschus esculentus*) plant growth, root morphological traits and physiological properties. *Saudi Journal of Biological Sciences*, 28(10), 5490-5499. <https://doi.org/10.1016/j.sjbs.2021.08.016>
- Joseph, S., Cowie, A. L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., Cayuela, M. L., Graber, E. R., Ippolito, J. A., Kuzyakov, Y., Luo, Y., Ok, Y. S., Palansooriya, K. N., Shepherd, J., Stephens, S., Weng, Z. H., & Lehmann, J. (2021). How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy*, 13(11), 1731-1764. <https://doi.org/10.1111/gcbb.12885>
- Karabay, U., Toptas, A., Yanik, J., & Aktas, L. (2021). Does Biochar Alleviate Salt Stress Impact on Growth of Salt-Sensitive Crop Common Bean. *Communications in Soil Science and Plant Analysis*, 52(5), 456-469. <https://doi.org/10.1080/00103624.2020.1862146>
- Keskin, S. O., Ali, T. M., Ahmed, J., Shaikh, M., Siddiq, M., & Uebersax, M. A. (2022). Physico-chemical and functional properties of legume protein, starch, and dietary fiber—A review. *Legume Science*, 4(1), e117. <https://doi.org/10.1002/leg3.117>
- Khan, M. N., Lan, Z., Sial, T. A., Zhao, Y., Haseeb, A., Jianguo, Z., Zhang, A., & Hill, R. L. (2019). Straw and biochar effects on soil properties and tomato seedling growth under different moisture levels. *Archives of Agronomy and Soil Science*, 65(12), 1704-1719. <https://doi.org/10.1080/03650340.2019.1575510>

- Kul, R., Arjumend, T., Ekinçi, M., Yildirim, E., Turan, M., & Argin, S. (2021a). Biochar as an organic soil conditioner for mitigating salinity stress in tomato. *Soil Science and Plant Nutrition*, 1-14. <https://doi.org/10.1080/00380768.2021.1998924>
- Kul, R., Ekinçi, M., Turan, M., & Yildirim, E. (2021b). Impact of Biochar on Growth, Physiology and Antioxidant Activity of Common Bean Subjected to Salinity Stress. *Global Journal of Botanical Science*, 9, 8-13.
- Lalay, G., Ullah, S., & Ahmed, I. (2022). Physiological and biochemical responses of *Brassica napus* L. to drought-induced stress by the application of biochar and Plant Growth Promoting Rhizobacteria. *Microscopy research and technique*, 85(4), 1267-1281. <https://doi.org/10.1002/jemt.23993>
- Lee, X., Yang, F., Xing, Y., Huang, Y., Xu, L., Liu, Z., Holtzman, R., Kan, I., Li, Y., Zhang, L., & Zhou, H. (2022). Use of biochar to manage soil salts and water: Effects and mechanisms. *Catena*, 211, 106018. <https://doi.org/10.1016/j.catena.2022.106018>
- Malhi, G. S., Kaur, M., Kaushik, P., Alyemeni, M. N., Alsahli, A. A., & Ahmad, P. (2021). Arbuscular mycorrhiza in combating abiotic stresses in vegetables: An eco-friendly approach. *Saudi Journal of Biological Sciences*, 28(2), 1465-1476. <https://doi.org/10.1016/j.sjbs.2020.12.001>
- Malik, A., Gul, S., Buriro, A. H., Kakar, H., & Ziad, T. (2022). Particle Size of Biochar as Co-composted Fertilizer: Influence on Growth Performance of Lettuce and Concentration of Bioavailable Soil Nutrients under Salinity Stress Conditions. Preprint -022010337 (doi: 10.20944/preprints202201.0337.v1).
- Mannan, M. A., Halder, E., Karim, M. A., & Ahmed, J. U. (2016). Alleviation of adverse effect of drought stress on soybean (*Glycine max.* L.) by using poultry litter biochar. *Bangladesh Agronomy Journal*, 19(2), 61-69. <https://doi.org/10.3329/baj.v19i2.31854>
- Mannan, M. A., Mia, S., Halder, E., & Dijkstra, F. A. (2021). Biochar application rate does not improve plant water availability in soybean under drought stress. *Agricultural Water Management*, 253, 106940. <https://doi.org/10.1016/j.agwat.2021.106940>
- Moradi, S., Rasouli-Sadaghiani, M.H., Sepehr, E., Khodaverdilo, H., & Barin, M. (2019). Soil nutrients status affected by simple and enriched biochar application under salinity conditions. *Environ Monit Assess* 191, 257. <https://doi.org/10.1007/s10661-019-7393-4>
- Mulcahy, D. N., Mulcahy, D. L., & Dietz, D. (2013). Biochar soil amendment increases tomato seedling resistance to drought in sandy soils. *Journal of arid environments*, 88, 222-225. <https://doi.org/10.1016/j.jaridenv.2012.07.012>
- Nguyen, B. T., Dinh, G. D., Nguyen, T. X., Nguyen, D. T. P., Vu, T. N., Tran, H. T. T., Thai, N. V., Vu, H., & Do, D. D. (2022). The Potential of Biochar to Ameliorate the Major Constraints of Acidic and Salt-Affected Soils. *Journal of Soil Science and Plant Nutrition*, 1-11. <https://doi.org/10.1007/s42729-021-00736-1>
- Nikpour-Rashidabad, N., Tavasolee, A., Torabian, S., & Farhangi-Abriz, S. (2019). The effect of biochar on the physiological, morphological and anatomical characteristics of mung bean roots after exposure to salt stress. *Archives of*

- Biological Sciences, 71(2), 321-327. <https://doi.org/10.2298/ABS181005014N>
- Obadi, A., AlHarbi, A., Abdel-Razzak, H., & Al-Omran, A. (2020). Biochar and compost as soil amendments: effect on sweet pepper (*Capsicum annuum* L.) growth under partial root zone drying irrigation. *Arabian Journal of Geosciences*, 13(13), 1-12. <https://doi.org/10.1007/s12517-020-05529-x>
- Parkash, V., & Singh, S. (2020). Potential of biochar application to mitigate salinity stress in eggplant. *HortScience*, 55(12), 1946-1955. <https://doi.org/10.21273/HORTSCI15398-20>
- Rengasamy, P. (2006). World salinization with emphasis on Australia. *Journal of Experimental Botany*, 57(5), 1017–1023. <https://doi.org/10.1093/jxb/erj108>
- Safaei Khorram, M., Fatemi, A., Khan, M. A., Kiefer, R., & Jafarnia, S. (2018). Potential risk of weed outbreak by increasing biochar's application rates in slow-growth legume, lentil (*Lens culinaris* Medik.). *Journal of the Science of Food and Agriculture*, 98(6), 2080-2088. <https://doi.org/10.1002/jsfa.8689>
- Sarkar, S., Khatun, M., Era, F. M., Islam, A. K. M., Anwar, M., Danish, S., Datta, R., & Islam, A. K. M. (2021). Abiotic stresses: Alteration of composition and grain quality in food legumes. *Agronomy*, 11(11), 2238. <https://doi.org/10.3390/agronomy11112238>
- She, D., Sun, X., Gamareldawla, A. H., Nazar, E. A., Hu, W., Edith, K., & Yu, S. E. (2018). Benefits of soil biochar amendments to tomato growth under saline water irrigation. *Scientific Reports*, 8(1), 1-10. <https://doi.org/10.1038/s41598-018-33040-7>
- Shimira, F., & Taşkın, H. Current progress on the responses of eggplant to ultra-low temperatures during production. *Horticultural Studies*. <https://doi.org/10.16882/hortis.1108342>
- Shimira, F., Uğur, S., Özdemir, Ş. M., & Mendi, Y. Y. (2021). Future and Prospect use of Pyrethrum (*Chrysanthemum cinerariifolium*) as Part of the Integrated Pest and Disease Management (IPDM) Tool in Turkey. *Turkish Journal of Agriculture-Food Science and Technology*, 9(1), 150-158. <https://doi.org/10.24925/turjaf.v9i1.150-158.3771>
- Smyth, S. J. (2022). Contributions of Genome Editing Technologies Towards Improved Nutrition, Environmental Sustainability and Poverty Reduction. *Frontiers in Genome Editing*, 15. <https://doi.org/10.3389/fgeed.2022.863193>
- Sofy, M., Mohamed, H., Dawood, M., Abu-Elsaoud, A., & Soliman, M. (2021). Integrated usage of *Trichoderma harzianum* and biochar to ameliorate salt stress on spinach plants. *Archives of Agronomy and Soil Science*, 1-22. <https://doi.org/10.1080/03650340.2021.1949709>
- Taqdees, Z., Khan, J., Kausar, S., Afzaal, M., Akhtar, I., & Hussain, S. (2022). Silicon and zinc nanoparticles-enriched miscanthus biochar enhanced seed germination, antioxidant defense system, and nutrient status of radish under NaCl stress. *Crop and Pasture Science*. <https://doi.org/10.1071/CP21342>
- Tian, Z., Wang, J. W., Li, J., Han, B. (2020). Designing Future Crops: challenges and strategies for sustainable agriculture. *The Plant Journal*, 105(5), 1165-1178. <https://doi.org/10.1111/tpj.15107>

- Wang, L., Ok, Y. S., Tsang, D. C., Alessi, D. S., Rinklebe, J., Mašek, O., Bolan, N. S., & Hou, D. (2022). Biochar composites: Emerging trends, field successes and sustainability implications. *Soil Use and Management*, 38(1), 14-38. <https://doi.org/10.1111/sum.12731>
- Yaashikaa, P. R., Kumar, P. S., Varjani, S., & Saravanan, A. (2020). A critical review on the biochar production techniques, characterization, stability and applications for circular bioeconomy. *Biotechnology Reports*, 28, e00570. <https://doi.org/10.1016/j.btre.2020.e00570>
- Yang, A., Akhtar, S. S., Li, L., Fu, Q., Li, Q., Naeem, M. A., He, X., Zhang, Z., & Jacobsen, S. E. (2020). Biochar mitigates combined effects of drought and salinity stress in quinoa. *Agronomy*, 10(6), 912. <https://doi.org/10.3390/agronomy10060912>
- Yao, R. J., Li, H. Q., Yang, J. S., Wang, X. P., Xie, W. P., & Zhang, X. (2022). Biochar Addition Inhibits Nitrification by Shifting Community Structure of Ammonia-Oxidizing Microorganisms in Salt-Affected Irrigation-Silting Soil. *Microorganisms*, 10(2), 436. <https://doi.org/10.3390/microorganisms10020436>
- Yao, R., Li, H., Yang, J., Zhu, W., Yin, C., Wang, X., Xie, W., & Zhang, X. (2022)combined). Combined application of biochar and N fertilizer shifted nitrification rate and amoA gene abundance of ammonia-oxidizing microorganisms in salt-affected anthropogenic-alluvial soil. *Applied Soil Ecology*, 171, 104348. <https://doi.org/10.1016/j.apsoil.2021.104348>
- Yildirim, E., Ekinici, M., & Turan, M. (2021). Impact of Biochar in Mitigating the Negative Effect of Drought Stress on Cabbage Seedlings. *Journal of Soil Science and Plant Nutrition*, 21(3), 2297-2309. <https://doi.org/10.1007/s42729-021-00522-z>
- Yu, Y., Zhao, C., Zheng, N., Jia, H., & Yao, H. (2019). Interactive effects of soil texture and salinity on nitrous oxide emissions following crop residue amendment. *Geoderma*, 337, 1146-1154. <https://doi.org/10.1016/j.geoderma.2018.11.012>
- Zhang, Y., Ding, J., Wang, H., Su, L., & Zhao, C. (2020). Biochar addition alleviate the negative effects of drought and salinity stress on soybean productivity and water use efficiency. *BMC Plant Biology*, 20(1), 1-11. <https://doi.org/10.1186/s12870-020-02493-2>



## CHAPTER 6

### ANTHURIUM BREEDING BY CLASSICAL AND BIOTECHNOLOGICAL METHODS

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## 1. INTRODUCTION

Anthurium is the most popular genus with the Araceae Family, which is a monocotyledonous and epiphytic ornamental plant (Castro et al., 2004). By discovering many new species especially in the Andes of Western South America, more than 1690 species were named and described today (Croat, 2015).

Although there seem to be different chromosome numbers in *Anthuriums*, the most common chromosome number is  $2n=30$  (Sheffer&Kamemoto 1976; Sheffer&Croat 1983).

**Table 1:**Taxonomy

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Monocotyledonae
Order	Arales
Family	Araceae
Genus	Anthurium

Its homeland is considered to be tropical regions of Central and South America, Colombia, Costa Rica, and Ecuador (Herk et al., 1998). The first known species of this genus owes its scientific name to the Austrian physician and botanist Karl von Scherzer, who discovered the first species of *Anthurium scherzerianum* in Costa Rica in 1850 and introduced it to Europe (Higaki et al., 1972).

The name *Anthurium* comes from the Greek. Combining the words Anthos = flower and Oura = tail, and it is called tail flower = tailed flower. The genus *Anthurium* called as “Flamingo flower” in our country and in some countries; “Cresto de Gallo” in America; “Bullshead” in China; “Tailflower” in America and “Lak-Anthurium” (Lacquer flower) = varnished flower in the

Netherlands. 40% of the production in the Netherlands consists of red varieties. Among the red varieties produced and sold at auctions, more than 95% of them are tropical varieties (Herk et al., 1998). *Anthurium andraeanum*, is grown traditionally for cut flowers for its large spathes and showy red, orange, pink or white colours (Henny et al., 1999) (Figure 1). This spathe has an enchanting beauty and not only its flowers, but also its aesthetic leaves are used as cut greens in arrangements. It has also managed to attract people's attention as a potted ornamental plant. Anthurium has always been in demand in the ornamental plants market. The volume of Anthurium sales is ranked second in the world, after orchids (Rikken, 2010; Hua, 2014).



**Figure 1 :** General view of *Anthurium*

## **2. ECOLOGICAL DEMANDS**

### **2.1. Light**

*Anthuriums* do not like direct sunlight and are grown in 73% and 80% shade. Shade is provided by tree ferns or natural vegetation with wood or lathe or polypropylene mesh. Excess light adversely affects the plant and requires 80% or more shade for best spathe colour development (Kunisaki, 1982; RA

Criley, 1988). If the plant is exposed to direct sunlight, the leaves will be damaged and may cause death of the plant.

## 2.2. Temperature

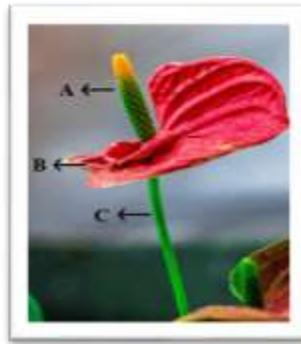
Since *Anthurium* is a tropical plant, they do not like extremely hot and extremely cold environments. The desired temperature in *anthurium* cultivation is approximately 26°C during the day and 16°C at night.

## 2.3. Irrigation

Considering that the natural habitats of *Anthuriums* are tropical regions; good drainage should be provided and irrigation should be done by moistening the soil surface in a small amount. *Anthuriums* are reported to be sensitive to sodium chloride, suffering economic reductions when irrigated by water of less than 0.6 mScm EC (Sonneveld and Voogt, 1983).

## 3. FLOWER STRUCTURE AND POLLINATION BIOLOGY OF ANTHURIUM

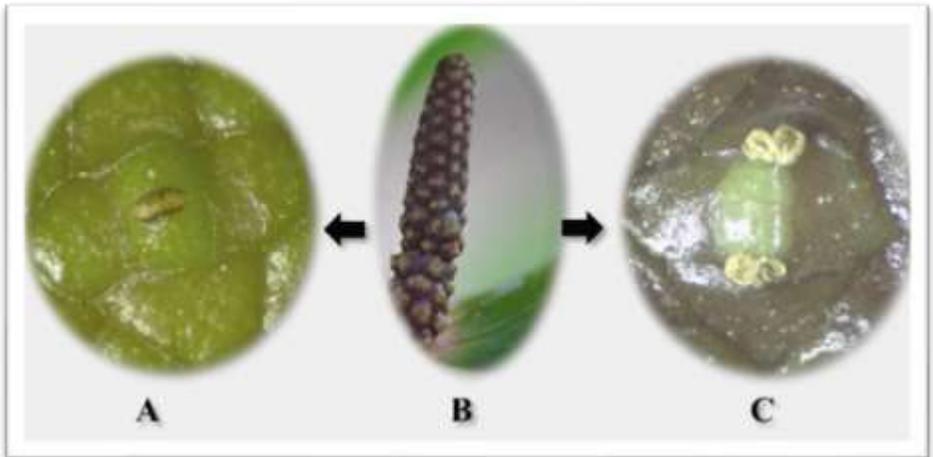
The term "flower" in *Anthuriums* refers to the spathe, spadix and peduncle (flower stalk) (Figure 2). The spathe of *Anthurium* is actually a modified bract that carries the erumpent cylindrical inflorescence rachis, which is called the spadix (Higaki et al., 1984; Vern,2004).



**Figure 2:** Anthurium Flower (A.Spadix, B. Spathe, C.Flowerstalk)

Approximately 150-300 flowers arrange helically on the spadix (Higaki et. al 1984). The flower structure of Anthuriums is hermaphrodite. Since the flower is protogynous, the stigmas protrude through the tepals and they become receptive, dehydrate and shrink at first. Then the anthers emerge, dehisce and shed their pollens. The time span between the receptivity of stigma and anther dehiscence is almost a week (Higaki et al., 1984). Therefore, foreign pollination occurs in Anthuriums (Figure 3).

Like all members of the Araceae, Anthuriums are entomophilous (Grayum, 1990; Franz, 2007).



**Figure 3:** Flower structure of *Anthurium* (A. Female phase, B. Spadix, C. Male phase)

#### **4. PROPAGATION METHODS OF ANTHURIUM**

Anthuriums are propagated by seed, cutting and tissue culture (Murguía, 1996).

##### **4.1. Propagation**

Seeds, collected from healthy plants, are planted in viols. After sowing, the seeds should remain in 75-80% shade in order to germinate immediately. By this way, the plantlets can be planted in 4 to 6 months. Flowering of the

seedlings can be expected in 15 months after planting, but most seedlings usually begin to bloom between 30 and 36 months. Growing Anthurium plants from seed is a lengthy process. It may take nearly 3 years from seed to bloom formation (Hikagi et al., 1995).

#### **4.2. Cutting**

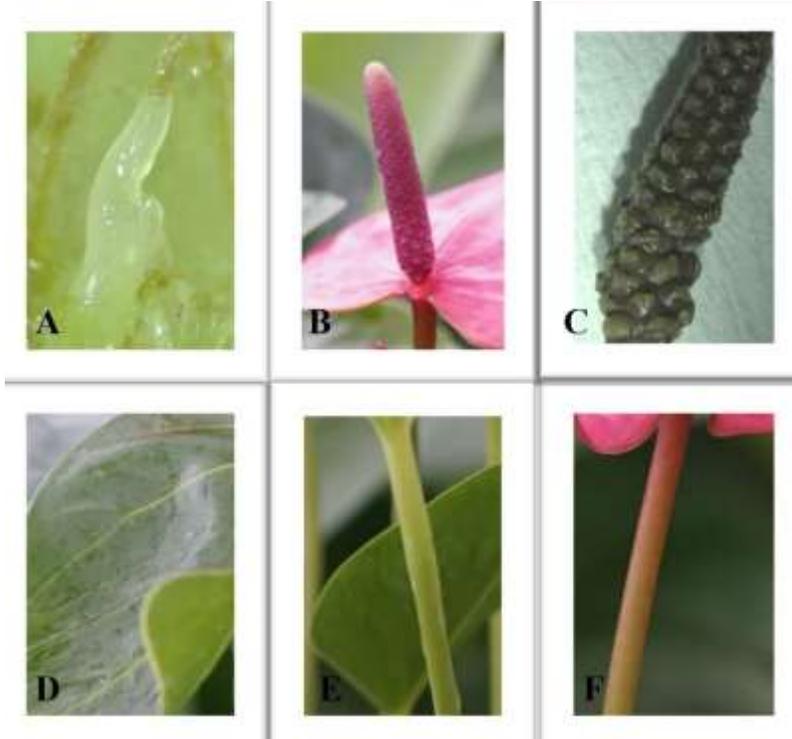
Vegetative propagation is the asexual method of propagation that insures the offsprings to be identical with their parent. In order to increase a particular cultivar the plants grow until some roots have developed on the stem. Then the rooty top is removed for producing a new plantlet. The remaining base of the stem will develop two or more side shoots (suckers). Repeating of this procedure ensures propagating large numbers of plants. The tendency of a plant to produce suckers is not only inherent in the cultivar but also influenced by its growing environment. In order to encourage new shoot production, the stems may be placed in damp media. However, with this method, reproduction of the plant is limited and a large number of plants cannot be obtained (Hikagi et al., 1972).

#### **4.3. Tissue Culture**

In seed production, it is not preferred commercially due to negative reasons such as genetically heterozygosity of the seed, low yield of the plant from the seed and long-term plant formation. Propagation by seed is preferred by breeders and hobby breeders. Although steel and separation methods are sufficient for hobby cultivation in Anthurium production, they are not considered sufficient for commercial production. Therefore, *in vitro* techniques are used for disease and pest-free production, clonal and mass production. Clonal production, clean plantlets and the possibility of mass production are the main advantages of tissue culture. Since Anthurium is a commercially valuable plant, many *in vitro* methods have been tried (Figure 4). The first tissue culture studies on Anthurium started in the mid-1970s (Pierik et al. 1974) and continued with different researchers such as (Pierik et al., 1974), (Eapen and Rao 1985), (Trujillo et al., 2000), (Mahanta and Paswan 2001), (Reddy et al., 2011), (Farsi et al., 2012), (Thockhom & Maitra 2017), (Oo, et al., 2019) (Table 2). Because of the Anthurium is a commercially important species and there are many varieties and genotypes, propagation protocols are still being developed and new ones are being added.

❖ *In vitro* methods used in Anthurium

- Somatic Embryogenesis
- Micropropagation
- Meristem Culture
- Organogenesis



**Figure 4:** Different Explant Types Used in Tissue Culture; (A.Meristem, B.Spathe, C.Spadix, D.Leaf, E.Petiole, F.Flowerstalk)

**Table 2:** Tissue culture studies at Anthurium

Genus	Explant	Regeneration type	Media	Growth Regulator	Researcher
A. Andraeanum	Leaf	Somatic Embryogenesis	(Pierik) Modified Murashige & Skoog's medium	PBA	Pierik et al. 1974
A. Patulum	Leaf, Spathe, Petiol	Callus Culture Shot and Root Regeneration	Modified Murashige & Skoog's medium	2,4D - Kinetin-Zeatin-2ip	Eapen and Rao 1985
Sonate	Leaf	Callus culture	Modified Murashige & Skoog's medium	Ba- 2,4D	Trujillo et al. 2000
Agnihorti	Axillary buds	Shoots Regeneration	Murashige and Skoog's medium	BAP - IAA	Mahanta and Paswan 2001
A. digitatum unspecified var.	Leaf	Callus, shoots, rooted	<sup>1/2</sup> Murashige & Skoog's medium	Ba- 2,4D-NAA	Reddy et al 2011
A. Andraeanum cv. Terra	Leaf	Callus & Organogenesis	Murashige & Skoog's medium	2,4-D BAP	Farsi et al. 2012
A. andraeanum cv. Jewel	Leaf	Callus & Shoots	Murashige & Skoog's medium	2,4D-NAA-BAP-TDZ	Thockhom & Maitra 2017
A. andraeanum Lind	Leaf & Petiol	Callus, Shoots & Roots	Murashige & Skoog's medium	2,4D-BAP	Oo, et al. 2019

In many studies in the literature, it has been observed that different combinations and concentrations of auxin and cytokinin are used. Since genotype is an important factor in regeneration, it can be said that the reason for these differences is genotype and breed differences. Anthuriums have mass propagation possibilities by using clean starting materials taken.

## **5. PLANT BREEDING**

Plant breeding began with the first humans by selecting those with superior characteristics, so the first breeding method was selection breeding. This process, which started with the domestication of wild species, continues today with same way. The Plant Hybridization Experiments carried out by Gregor Mendel in 1866 who became the turning point of plant breeding and genetics and guided the work done today. In order to perform hybridization breeding in a hermaphrodite plant, first of all, the male organs (anthers) on the flower of the female parent should be emasculated and the female organ (pistil) should be left alone. Then, the desired male parent pollen is brought on the pistil of female parent and by this way pollination is carried out.

### **5.1. Anthurium Breeding**

Anthurium breeding is gaining importance as this genus has always had an important place in the floriculture market. Classical and biotechnological methods are used in plant breeding, but multidisciplinary studies give better and shorter results.

#### **5.1.1. Classical Method**

The basis of classical cross breeding is emasculation and controlled pollination. However, there is dichogamy in flowers of Anthuriums. In other words, male and female organs mature at different periods. In this context, Anthuriums are in the group of plants with protogynous feature. In Anthurium flowers, the female organs become receptive first. The maturation of the female organs is from the bottom to up and takes quite a long time. The stigma receptivity can be easily recognized by the velvety and shiny structure of the inflorescences due to wetness. All of the stigmas are receptive and after the completion of the female phase, the stigmas dehydrate and turn to brown. After stigma uptake is completed, anthers are formed, again starting from the bottom. At this stage, the anthers mature and release pollen. Pollens of Anthiriums are very small. Although very small flowers with cluster structure in anthuriums seem to create difficulties in emasculation and hybridization for

reproduction, the absolute dichogamy–protogynous- structure of the plant eliminates the need for emasculation and provides easy pollination procedure in the stage when the pistil is receptive (Grayum, 1990). However, since pollen release is very low, pollen can be transferred to the receiving stigmas with the help of our finger or a short-bristled brush.

At this stage, the gradual structure of the flowers, that is, one flower in the female stage and the other in the male stage, will facilitate the hybridization processes.

### **5.1.2. Points to Consider in *Anthurium* Hybridization**

In order to obtain sufficient seeds in *Anthuriums*, the flowers need to be pollinated many times. The pistils of the flowers mature within a week or two, but each pistil on the spadix remains receptive (can be pollinate) for only 1 day. In this case, the obtained pollen should be brought to the stigmas of the pistils at several days intervals and each flower should be pollinated several times.

### **5.1.3. Development of F1 Plants**

After the seeds obtained after 6 to 8 months (Henny et al., 2008) , they are germinated under suitable conditions, a long period of 3 years is required for flower formation (Hikagi et al., 1995). Good maintenance and greenhouse conditions may be used to shorten this period in natural conditions.

## **5.2. Biotechnological Methods**

Biotechnological breeding methods are used to support classical breeding methods and to ensure the breeding program to reach results in a shorter time.

Biotechnological Breeding methods ;

- ✓ Haploidization
- ✓ Somatic hybridization
- ✓ Marker assisted selection
- ✓ Somaclonal variation
- ✓ In vitro mutation

In addition to traditional plant breeding, new techniques are needed to improve plant varieties in the *Anthurium* plant. Therefore, for creating additional variation in a different way breeders may use mutation breeding. The application of ionizing radiation, chemical mutagens and somaclonal variation, which is one of the in vitro techniques, is quite common in the generation of genetic variation (Puchooa, D., 2005).

### 5.2.1. Haploidization

Haploidization; by making use of the gametes, which have half (n) of the normal chromosome number of a species is the production of plants that carries the gametic chromosome number of that species.

Breeders used haploidization method to save time and money by shortening the plant growing process while obtaining a pure line. Homozygous reproduction-folding is required for haploid plantlets regenerated from anthers *in vitro* to interact effectively. Colchicine is one of the most common mutagenic chemicals used *in vivo* and *in vitro*. (Castillo et al. , 2009) The first anther culture of *Anthurium* was tested by Custers (2004) with culturing the whole anther with filament in half MS medium.

### 5.2.2. Somatic Hybridization

Protoplast culture, one of the plant tissue culture techniques, is the isolation, culture and fusion (hybridization or fusion) of cells without a cell wall. The most important reason for using protoplast culture in plant breeding is that this method offers new opportunities in plant breeding. By using the somatic hybridization technique, desired characters of two different species are combined and a new species can be created (Anonymous, 1995, Espinosa et al. 1986, Harding and Millam, 2000; Kumlay et al., 2005;). In a study by Duquenne (2007), protoplast culture was performed on *Spathiphyllum wallisii* 'Alain' and *Anthurium scherzerianum* plants. It has been reported that as a result of protoplast culture, the cells develop up to the colony stage (Duquenne, et al., 2007).

### 5.2.3. Somaclonal Variation

The definition of somaclonal variation is appeared by cytological abnormalities, frequent qualitative and quantitative phenotypic mutations, sequence changes, and gene activation or silencing (Kaeppler et. al, 2000). This method is used to create breeding populations and to provide genetic diversity in plants with fertilization problems or difficulties in creating populations. Somaclonal variation is an undesirable situation during propagation, but it is an auxiliary biotechnological method that can be used in plant breeding.

### 5.2.4. Marker Assisted Selection

Molecular marker technology has led to the adoption of wide-ranging new applications to improve selection strategies in plant breeding. Molecular markers are DNA fragments associated with any gene region or gene region in the genome. Molecular markers are markers that reveal the DNA sequence

difference of different genotypes in various ways. The use of nucleic acid-based genetic markers in genome analysis is an area needed for breeders. By using these markers, varieties that are morphologically very close to each other can be distinguished and identified (Yorgancılar et al, 2015). Because of the process from seed to flowering in Anthurium takes about 3 years, shortening this long process by using marker assisted selection will ensure the breeders to select plants earlier, less labor, time and save cost.

### **5.2.5. *In Vitro* Mutation**

In order to induce useful phenotypic variations, mutagenic agents have been used for more than 70 years (Foster&Twel, 1996). Numerous mutant lines were isolated from many plants and these are used for plant research and crop cultivation (Evans, 1962). Besides traditional plant breeding methods, new techniques are required for Anthurium breeding. Mutation breeding like ionizing radiation and chemical mutagens in tissue culture may be proposed for creating variations. In Anthuriums, gamma radiation indicates both positive and negative mutations and could be used for further breeding programmes (Puchooa, 2005).

Gene mutations occur less frequently than chromosomal mutations, which include translocations, inversions, deletions, and omissions. Mutations affect parts of a gene, single base pairs, or groups. Alteration of base pairs or changes in their sequences can alter the primary gene product and ultimately lead to an altered phenotypic expression of one or more traits through a complex chain reaction of events.

For this reason, breeding studies using mutagenic chemicals can be used as an alternative to classical breeding methods.

## REFERENCES

- Collette, V. (2004). Anthuriumaristocracy. *NZ Gard J*, 7(1), 3-5.
- Criley, R. A. (1988, October). Culture and cultivar selection for anthurium in Hawaii. In *International Symposium on Protected Cultivation of Ornamentals in Mild Winter Climates 246* (pp. 227-236).
- Croat, T. B. (1983). A revision of the genus *Anthurium* (Araceae) of Mexico and Central America. Part I: Mexico and middle America. *Annals of the Missouri Botanical Garden*, 211-416.
- Croat, T. B. (2015). A review of studies of Neotropical Araceae. *Aroideana*, 38(1), 44-54.
- Custer, J. B. M. (2004). Preliminary research in anther culture of *Anthurium*. HAPLIN Report, (20), 1-6.
- DE CASTRO, A. C. R., RESENDE, L. V., GUIMARÃES, W. N. R., & LOGES, V. (2004). Uso de técnicas moleculares em estudo de diversidade genética em *Anthurium*. *Ornamental Horticulture*, 10(1/2).
- deLeal, A. T., & Daquinta, M. (1999). Micropropagación de variedades *Anthurium andraenum* de interés comercial. *Agrícola vergel: Fruticultura, horticultura, floricultura*, (216), 793-804.
- digitatum*, using leaf as explant. *Asian J. Pharm. Health Sci.* 1 (2), 70-74.
- EAPEN, S., & Rao, P. S. (1985). Regeneration of plants from callus cultures of *Anthurium patulum*. *Current Science*, 54(6), 284-286.
- Farsi, M., Taghavizadeh, Y. M., & Qasemimran, V. (2012). Micropropagation of *Anthurium andreanum* cv. Terra. *African Journal of Biotechnology*, 11(68), 13162-13166.
- Franz, N. M. (2007). Pollination of *Anthurium* (Araceae) by derelomine flower weevils (Coleoptera: Curculionidae). *Revista de biología tropical*, 55(1), 269-277.
- Henny, R. J. (1999). Red Hot'Anthurium. *Hort Science*, 34(1), 153-154.
- Henny, R. J., Chen, J., & Mellich, T. A. (2008). Tropical foliage plant development: Breeding techniques for *Anthurium* and *Spathiphyllum*. *EDIS*, 2008(5).

- Herk M., Van Koppen M., Smeding S., Van Der Elzen C.J., Van Rosmalen N., Van Dijk J., Lont A., Van Spingelen J., 1998. In: Anthura, B.V. Ed., Cultivation Guide Anthurium. Bleiswijk, Holland, 140 p
- HerK, M. Van /et.al./. Guía del cultivo del Anthurium. Editado en Anthura. BV. Holanda, 1998.
- Higaki, T., & Watson, D. P. (1972). Anthurium culture in Hawaii, University of Hawaii. *Service Circular*, (420), 20.
- Higaki, T., Lichty, J. S., & Moniz, D. (1995). Anthurium culture in Hawai'i.
- Higaki, T., Rasmussen, H. P., & Carpenter, W. J. (1984). A study of some morphological and anatomical aspects of Anthurium and reanum Lind. <https://www.cabi.org/isc/datasheetreport/7993> - Report date :
- Hua, X. (2014). Flower market research report in Chinese New Year in 2013. *China Flowers Hortic*, 13, 24-28.
- Mahanta, S., & Paswan, L. (2001). In vitro propagation of Anthurium from Axillary Buds. *Journal of Ornamental Horticulture*, 4(1), 17-24.
- Mendel, KG. (1866). Versuche über pflanzen-hybriden. Verhandlung des naturforschenden Vereins in Brunnfur, 4, 3-47.
- Murguia Gonzalez, J. (1996). Evaluation of growing media Anthurium in Amatlan de los Reyes, Ver. *Memorias Cientificas (Mexico)*.
- Oo, K. T., Htun, N. M., Htwe, M. Y., Mon, A. M., Htet, W. T., & Win, N. A. (2019). In vitro Propagation of Anthurium and reanum Linn.(White) via Indirect Organogenesis through the Use of Leaf Lamina and Petiole Explants. *Journal of Scientific and Innovative Research*, 8(3), 78-82.
- Puchooa, D. (2005). In vitro mutation breeding of Anthurium by gamma radiation. *International Journal of Agriculture and Biology*, 7(1), 11-20.
- Reddy, J.M., Bopaiah, A.K., Abhilash, M., 2011. In vitro micropropagation of Anthurium
- Sonneveld, C., & Voogt, W. (1983). Studies on the salt tolerance of some flower crops grown under glass. *Plant and Soil*, 74(1), 41-52.
- Thokchom, R., & Maitra, S. (2017). Micropropagation of Anthurium and reanum cv. Jewel from leaf explants. *Journal of Crop and Weed*, 13(1), 23-7.

- KUMLAY, A. M., & PEHLUVAN, M. PATATES (SOLANUM TUBEROSUM L.) ISLAHINDA PROTOPLAST KÜLTÜRÜYLE SOMATİK MELEZLEME.
- Duquenne, B., Eeckhaut, T., Werbrouck, S., & Van Huylenbroeck, J. (2007). Effect of enzyme concentrations on protoplast isolation and protoplast culture of *Spathiphyllum* and *Anthurium*. *Plantcell, tissue and organ culture*, 91(2), 165-173.
- Kaeppler, S. M., Kaeppler, H. F., & Rhee, Y. (2000). Epigenetic aspects of somaclonal variation in plants. *Plant gene silencing*, 59-68.
- YORGANCILAR, M., YAKIŞIR, E., & ERKOYUNCU, M. T. (2015). Moleküler Markörlerin Bitki Islahında Kullanımı. *Bahri Dağdaş Bitkisel Araştırma Dergisi*, 4(2), 1-12.
- Puchoa, D. (2005). In vitro mutation breeding of *Anthurium* by gamma radiation. *International Journal of Agriculture and Biology*, 7(1), 11-20.
- Foster, G.D. and D. Twell, 1996. *Plant Gene Isolation: Principles and Practice*. pp. 215-45. John Wiley and Sons Ltd.
- Evans, H.J., 1962. Chromosome aberrations induced by ionizing radiations. *Int. Rev. of Cyt.*, 13: 221-308

## CHAPTER 7

### BIOTECHNOLOGICAL STRATEGIES FOR THE MITIGATION OF ABIOTIC STRESS IN FRUIT CROPS UNDER SUSTAINABLE AGRICULTURE

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## **INTRODUCTION**

Global food production is decreased by extreme abiotic conditions like drought, extreme heat, salinity, etc. Among these, the stability of agro-ecosystems is significantly impacted by the increase in atmospheric temperature caused by natural and anthropogenic sources. According to the Intergovernmental Panel on Climate Change (IPCC), if anthropogenic sources continued to contribute to global warming in the current pattern there is a possibility that the average temperature of the earth may rise by 6.4 C. Thus, by the end of the 21st century, the melting of glaciers will expedite sea levels by approximately 59 cm. Climate changes increase the chances of natural disasters like flash floods, cyclones, storms, drought and weather patterns. Since agriculture is extremely climate reliant and sensitive to agro-climatic conditions, changes in temperature, moisture, and rainfall have a negative influence on agricultural plant growth (Hossain et al., 2021).

Stress is generally characterized as an outside factor that adversely affects the plant. In most cases, stress is analyzed in terms of crop production, biomass accumulation, plant survival, or the main assimilation pathways (CO<sub>2</sub> and mineral absorption), which are all indications of overall development. Different environmental parameters, like atmospheric temperature, might become stressful in a couple of minutes, while others, like some mineral nutrients, may take days or even months to become stressful. Waterlogging, high temperatures, cold and freezing stress, salinity stress, and flooding stress are the few different abiotic factors that have an impact on fruit development. Even though it is easier to look at each of these elements independently, many of them are interlinked. For instance, a deficiency of water is usually followed by salt in the root zone or excessive heat in the leaves. Cross-resistance, or reaction to one stress caused by adaptation to another, is common in plants. This behavior suggests that the resistance

mechanisms to diverse pressures have many similarities. Plants are subjected to severe environmental pressures throughout their life cycle that also have a detrimental influence on growth and have a substantial impact on crop yield. Fruit trees are perennial crops, therefore once they are established, and they are always susceptible to a range of stresses. It would be difficult for the fruit trees to recover if they were severely harmed by environmental pressures, which would result in reduced growth and less fruit production. Additionally, the detrimental impacts of stress may not only affect the current year's fruit yield but also continue across the subsequent year (s). Consequently, it is necessary to develop strategies for minimizing stress damage and enhancing stress resistance in fruit cultivars for long-term cultivation, which can be done through genetic engineering and various other biotechnological approaches (Bakshi et al., 2022)

## 1. ABIOTIC STRESS

Drought, salinity, temperature, and flooding are some examples of environmental stress factors that can seriously disrupt growth and production characteristics and reduces the value of fruits in tropical fruit plants. The reactions of plants to environmental stresses have been studied using a range of omics techniques (Zhuang, et al., 2014). In mango fruits, intrinsic tissue breakdown was found to be induced by high temperatures (Vasanthaiah et al., 2008). During exposure to heat stress at 44 °C as well as under chilling stress, fruits have demonstrated increased expression of senescence-related and abiotic genes (Khanum et al., 2020). Transcriptome studies contributed to the discovery of 18 *eIF* gene which activates in response to temperature stress, salt and osmotic stress (Li et al., 2019). Moreover, bHLH TF genes as well as WD40 protein families that regulate abiotic stress levels in papaya, mango and citrus, particularly citrus can give information to understand responses to saline, drought, alkaline, and freezing environmental stress (Salih et al., 2021).

Chilling stress in fruits not only causes physical damage to fruit but also significantly reduces the flavor. Chilling damage to the fruits, in addition to inflicting physical damage, can significantly impair fruit flavor. Chilling damage was generated by banana fruits storage at 5 C and 20 C, which affected the fruit morphology and decreased volatile synthesis (Zhu et al., 2018). Furthermore, correlative transcriptomic, as well as proteome profiling of lowered thermal treated bananas, helped in the discovery of MYB TFs as well as 12,462 cold stress-responsive lncRNAs (Liu et al., 2018). Parallel studies contributed to understanding the molecular mechanisms of citrus fruit that respond to decreased temperatures to maintain nutritional fruit value and extend lifespan (Yun et al., 2012). Cold temperature (10 C) boosted carotenoid biosynthetic gene expression (*CitPSY* and *CitVDE*) while decreasing catabolic expression of the gene (*CitNCED2* and *CitNCED3*), raising total carotenoid accumulation in citrus fruit sacs. Citrus fruits are extremely susceptible to salinity stress, and researchers have indicated genes involved in weakening of cell walls and stiffening in response to salinity, while alkaline stress has been linked to miRNAs and 28 PHAS genes (Wu et al., 2016). Assessment of drought-resistant and susceptible guava germplasm with SRAP along with ISSR markers has proven successful in evaluating drought-resistant germplines (Abouzaid et al., 2019). In guava, however, 40 putative microRNAs linked to saline conditions and their target fragments were discovered (Sharma et al., 2020). CpHSF, CpMYB, CpNAC, CpNFY-A, CpERF, and CpWRKY are transcription factors that regulate drought stress tolerance in papaya (Gamboa-tuz et al., 2018). Despite the lack of genetic knowledge on water stress stress-related processes in jackfruit, SSR markers developed and QTLs discovered for drought tolerant root features in mulberry can be used to investigate jackfruit genotypes because they demonstrated 79.25 percent cross-species replicability (Biradar et al., 2013). Drought stress

increased the levels of abscisic acid receptor PYL8, amino acid permease 8, and C2H2 zinc finger proteins in dragon fruits (Fan et al., 2014). The dragon fruit catalase gene (HuCAT3) and related proteins have been shown to play an important role in chloroplast and mitochondria metabolism under abiotic conditions (Nie et al., 2015).

### **1.1. Drought stress**

In fruit orchards, soil moisture has a significant impact on fruit size, production, and plant development. Under conditions of drought, the amount of water that is absorbed by the soil in fruit orchards is less than the amount that is lost via stomata thus suppressing cell growth. The plant's cells contract and cell walls disintegrate as its water content drops. The solutes inside the cells accumulate as the water loss increases and the cells continue to constrict. The plasma membranes thicken and constrict more. Turgor-dependent processes are especially susceptible to a water shortage as turgor reduction is the earliest substantial biophysical impact of water stress. Significant leaf development happens at night because plants that are dehydrated tend to become rehydrated at that time. On indeterminate plants, water stress restricts the both size and number of leaflets because it reduces the number of branches and their rate of growth. If the plants get water stressed after developing a significant leaf area, the leaflets will senesce and fall off. This leaf section modification is a significant enduring alteration that increases the plant's strength in water-stressed conditions (Fernandes et al., 2018). In most cases, water pressure can negatively affect both stomatal closure and photosynthesis in the leaf. Since stomatal closure limits transpiration as much as it decreases intercellular Co<sub>2</sub> levels, water usage efficiency may rise during the early phases of water stress. Photosynthesis is also inhibited when mesophyll cells are dehydrated. However, when stress increases, water utilization efficiency falls and inhibition by mesophyll cells becomes higher. The formation of a

thick cuticle, which reduces water loss in the epidermis, is a frequent physiological reaction to water stress (cuticular transpiration). Although a thicker cuticle reduces CO<sub>2</sub> absorption, leaf photosynthesis remains intact because epidermal cells beneath the cuticle are non-photosynthetic (Bakshi et al., 2022).

Drought is the most harmful abiotic stress that is reducing the production of fruits, vegetables and ornamental plants. The current situation of global warming and the improper raining system has enhanced the impact of drought stress on horticultural crops. Drought stress imparts morphological, physiological and metabolic changes in plants (Ahmad et al., 2021). Morphologically, drought stress alters root growth, leaf angle and leaf area. Whereas at the physiological level, osmotic adjustment, leaf water potential, transpiration rate, stomatal regulation and photosynthetic activities are affected (Ahmad et al., 2021). Drought stress affects the metabolic process of plants and may lead to the accumulation of a higher amount of free sugars, and free essential amino acids. Drought stress can be eliminated by the selection of appropriate genetic material and manipulation of drought tolerance or drought resistance genes in crop plants (Ahmad et al., 2015, 2018). In fruit plants, drought stress exerts some positive effects on fruit quality because it may cause higher sugar accumulation, organic acid formation and fruit maturation. However, this may depend on plant species, developmental stages and duration of drought stress.

Biotechnological approaches such as QTL mapping, Genome-wide association study, and gene transformation through different mediums have allowed plant biologists to develop the drought resistance germplasm. Transgenic plants displayed higher tolerance to drought, ABA-hypersensitive stomatal closing, an increase in leaf stomata and trichome density, and compact cuticle structures with a lower number of micro-channels (Pieczyński

et al., 2013). Drought resistance can be incorporated through the transformation of regulatory genes (Zinc finger proteins, NAC transcription factors, MYB transcription factors, Wax biosynthesis genes) and structural genes (proline, glycine betaine, trehalose, mannitol and LEA proteins). Drought tolerance genes have been achieved in tomatoes and apples (Szakiel et al., 2012; Qi et al., 2019; Diao et al., 2020). Molecular approaches have been used to identify the QTLs responsible for drought tolerance in different plant species (Rahman et al., 2011; Mace et al., 2012). Drought resistance cultivars may possess a higher cuticular wax load that may increase the shelf life of plants and reduce the transpiration rate from fruits. Higher wax accumulation along with drought resistance has been observed in apples (Qi et al., 2019) and tomatoes (Leide et al., 2007). Recently it has been reported that miRNAs-based genetic modification leads to develop the drought tolerance genotypes in horticultural crops. miRNAs have been identified that control  $\beta$ -diketone wax formation and minimize the water loss from the plant's surface and tolerate drought stress (Huang et al., 2017). Phytohormones play an important role to eliminate the effect of drought stress. Overexpression of ABA-related genes and TFs such as NAC TFs, and RD26 shows high ABA sensitivity and causes up-regulate of the drought-responsive genes (Fu et al., 2017). Table 1 illustrates the some drought genes and traits identified in fruit crops.

**Table 1.** Drought tolerance/resistance genes expressed in different horticultural crops.

<b>Fruit species</b>	<b>Crop</b>	<b>Gene/TF</b>	<b>Trait</b>	<b>References</b>
Tomato		<i>CBF/NHX1/DREB1</i>	Drought tolerance	(Solankey et al., 2015)
Tomato		<i>HsfA1a</i>	Drought tolerance	(Wang et al., 2015)
Tomato		<i>SISHN1</i>	Cuticular wax accumulation	(Al-Abdallat et al., 2014)

Tomato	<i>GDSL1</i>	Drought tolerance via cutin deposition	(Girard et al., 2012)
Tomato	<i>sly-miR159</i>	Drought tolerance	(López-Galiano et al., 2019)
Tomato	<i>MdPIP1;3</i>	Drought tolerance	(Wang et al., 2017)
Apple	<i>MdSHN3</i>	Cuticular wax biosynthesis	(Lashbrooke, Aharoni & Costa, 2015)
Banana	MaAQPs	Abiotic stress tolerance	(Hu et al., 2015)
strawberry	<i>FaPIP1</i>	Water permeability	(Alleva et al., 2010)

### 1.1.1. Response of fruit plants to drought stress and its management

Multiple processes seem to be involved in the plant response caused by water constraints. Stomatal closure, which decreases water loss and controls plant water content, is one of the most prevalent methods. Leaf moisture content has been used to identify plant water crisis. It is thought that hormonal signaling from the roots has a role in the early drought response. In response to drought stress, Zhu et al. (2004) examined the leaf water capacity and intrinsic cytokinins in the xylem sap of early apple trees administered with or without paclobutrazol. The findings demonstrated that dryness considerably reduced the plants' moisture retention, but paclobutrazol treatment greatly decreased water holding capacity. In comparison to the drought treatment solely, the paclobutrazol-treated plants often had a high proportion of zeatin riboside. This could happen because of paclobutrazol ability to prevent cytokinin oxidation. Progress has been made in applications of molecular breeding approaches to increase the disease and stress tolerance of various fruit crops. To do this, the efficacy of a ferritin gene from *Medicago sativa* (MsFerr) was examined in grapes. It is suggested that increasing the total ferritin content protects plant cells against oxidative damage caused by a

variety of stressors by isolating the intracellular iron implicated in the formation of particularly reactive hydroxyl group radicals via Fenton-reaction. The current regeneration and transformation methods have been enhanced. The use of 1 M benzyl adenine increased the proportion of regenerated plants (BA). Plant reproduction was improved by keeping plant material on "half MS" media without a selective agent (after two years of selection) and cutting aberrant embryos underneath the hypocotyl. The separately regenerated transformants were examined using PCR, qPCR, and Western blot, and they were employed in a variety of abiotic stress tolerance studies (Parmar et al., 2017). FaOLP2, a strawberry genomic clone encoding an osmotin-like protein (OLP) gene, was extracted and sequenced. FaOLP2 is projected to encode a 229-amino acid precursor protein, and its sequence has substantial similarities with several other OLPs. FaOLP2 is a multi-gene family, according to a genomic DNA hybridization study. Real-time PCR was employed to examine the expression of FaOP2 in several strawberry parts. The results reveal that FaOLP2 was expressed at differing stages in foliage, crowns, rhizome, green fruits, and mature red fruits. Furthermore, the activity of FaOLP2 under various abiotic stressors was studied at various periods. Within 2-6 hours after treatment, all three abiotic stressors studied, abscisic acid, salicylic acid, and mechanical injury, induced a considerable induction of FaOLP2. Furthermore, salicylic acid stimulated FaOLP2 more strongly than abscisic acid or mechanical damage. The favorable responses of FaOLP2 to the three abiotic stressors revealed that strawberry FaOLP2 might contribute to defending against osmotic-related environmental factors and may potentially play a role in plant defense against infections (Meena et al., 2017).

## **1.2. Chilling and freezing stress**

Cold damage is most common in tropical or subtropical species. Freezing injury, on the other hand, happens when the temperature of the water falls below the freezing point. Plant leaves damaged by cold display decreased photosynthesis and glucose translocation, delayed respiration, decreased protein synthesis, and accelerated breakdown of existing proteins (Zhou et al., 2020). All these reactions are most likely based on a common underlying mechanism that involves membrane function loss upon chilling. When tissue is naturally chilled, ice usually forms initially within the intercellular gaps and xylem vessels, where it may rapidly disseminate. Resistant plants are not harmed by this ice deposition, and the tissues restore completely when warmed. When plants are subjected to freezing conditions for a lengthy period, the formation of extracellular ice crystals causes the migration of water vapors from the fruiting bodies to the extracellular ice, resulting in severe dehydration (Ahn et al., 2012). Ice nucleation is the mechanism by which these dozens of ice molecules begin to create a stable ice crystal. Ice nucleators are big polysaccharides or proteins that aid in the production of ice crystals. Ice crystals begin to develop in plant cells from intrinsic ice nucleators, and the subsequent rather large internal ice crystals cause substantial cell damage and seem to be typically harmful (Duan et al., 2012).

### **1.2.1. Management of chilling/freezing stress**

Crop protection efforts trace back at least 2000 years when Roman farmers conserved grapes by burning dispersed bodies of dead vines and peelings. Several distinct freeze protection technologies have arisen over the last 100 years as a result of major research efforts devoted to lowering freezing damage in horticulture crops. Significant progress has been made in identifying genetic control of cold hardiness as well as tolerance to other abiotic stimuli during the last two decades. The first focus of the research was

on extracting and characterizing cold-regulated (cor) genes, which has been followed by the identification of cold-induced transcriptional regulators and the analysis of cold-induced alterations in complete genomes employing microarray technologies. Metabolomics and proteomics have also revealed the physiology and biochemistry of cold adaptation. Cold tolerance is a complicated feature in fruit trees and woody plants in particular, and a comprehensive view of the physiology of chilling tolerance is required if biotechnology is to be employed effectively to increase environmental stress resistance (Liu, et al., 2018).

Understanding the regulatory oversight of HSP gene transcription, as well as the discourse of their activity are essential prerequisites for developing transgenic crops capable of increasing HSP accumulation, particularly through HSTF upregulation, intending to improve tolerance to chilling injury. The observed ability of HSP to scavenge ROS and their interference in publicizing antioxidant systems, particularly by inoculation of APX gene expression and an increase in Gsh content, their contribution in boosting proline biosynthetic genotypes leading to proline deposition, and eventually their input to RFO accumulation by controlling galactinol and raffinose biosynthesis, are all biological events in which HSP is observed to contribute in the protection of fruits and vegetables. Modulation of HSP gene expression opens several possibilities for reducing post-harvest chilling damage. It would open up new possibilities for using biotechnological technologies to increase thermophilic temperature resistance and lengthen the time of chilled storage in fruits, horticultural crops, and vegetables that are susceptible to chilling damage (Aghdam et al., 2015).

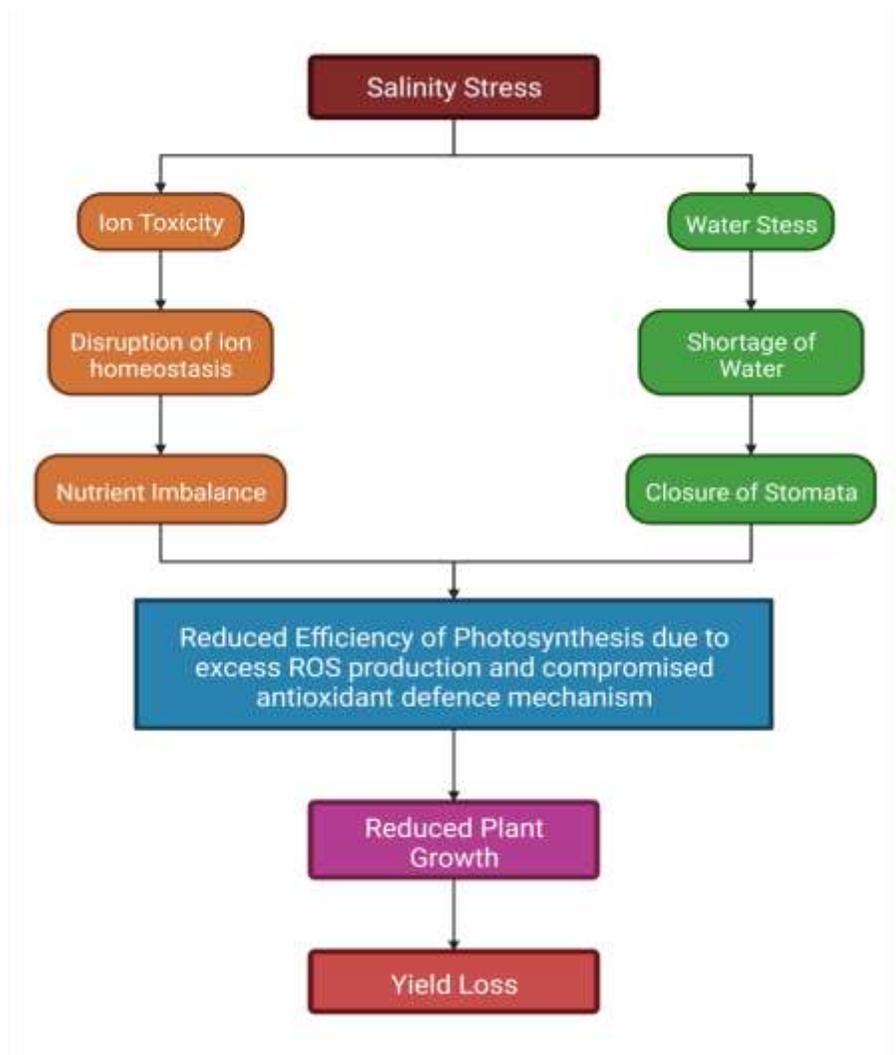
### **1.3. Salinity stress**

Soil salinity is a major issue in arid and sub-arid areas, where plants are subjected to high temperatures and severe water shortages during the dry

season. Salts tend to build up in the soil due to high evaporation rates and insufficient ion leaching in these climatic conditions, a problem that is frequently exacerbated by saline irrigation water in agricultural production areas (Levy et al., 2010). Saline water can arise as a consequence of coastal seawater pollution of fresh groundwater supplies or draining wastewater from irrigated areas. There are fewer incidents of salinity caused by the geological formations of soils. Salinity is one of the key restriction factors in fruit tree yield (Negrão et al., 2017). In addition to immediately harming plants, sodic soils' high Na<sup>+</sup> concentrations also deteriorate the soil's structure by reducing water vapor permeability. The bigger the EC and the lesser the osmotic potential of water, the greater the salt concentration. In semi-arid and desert areas, irrigation water is of poor quality.

High-salt stress affects plants in multiple ways, such as ion toxicity, nutritional disorders, alteration of metabolic processes, oxidative stress, genotoxicity, membrane disorganization, reduction of cell division and expansion as well as water stress. The accumulation of too much salt in the soil eventually leads to inhibition of plant growth and leads to plant death. Globally, no other toxin is as harmful to the growth of plants as salt. Salinity is considered an alarming phenomenon because it reduces agricultural production and results in the reduction of per hectare yield. It is estimated that 20% of all arable land and 50% of all irrigated lands are affected by salinity, which can decrease the production potential of the plant. It is supposed that the increased level of salt in the soil is due to low-quality irrigation water, and the use of saline solutions. High-salt stress can impact fruit plants in many ways, such as ion toxicity, genotoxicity, nutritional disturbances, membrane fragmentation, metabolic processes, cell proliferation, oxidative stress, reduced cell division, and water stress as shown in Figure 1 (Ijaz et al. 2018). Plants have developed the following mechanism to retain their growth and

development during salt stress. This mechanism has four major steps, including (a) avoidance: to avoid contact with salts present in the soil, (b) escape: alteration of the life-cycle, (c) recovery: to enhance the vegetative growth potency, and (d) tolerance: invalidating the effects of salinity stress (Ijaz et al. 2020).



**Figure 1:** Impacts of salinity stress on fruit trees.

Investigating the physiological and genetic basis of salt tolerance in fruit crops is vital to finding more effective solutions to the practical complications caused by salinity. It is possible to reduce production losses caused by high salinity stress by grafting salt-sensitive varieties on rootstocks that are resistant to salt. This approach can also produce plants with the good shoot and root characteristics. However, salt tolerance is a polygenic phenomenon consisting of a complex network of physiological and molecular processes. Therefore, the assessment of physiological characteristics by various biotechnological techniques is considered a more effective markers for assessing salt tolerance in many fruit crops (Ahmad and Anjum 2020). Effective use of limited water resources becomes even more necessary due to the rapid increase in agriculture in arid and semi-arid regions. Especially in arid and semi-arid environments, the presence of high amounts of soluble salts in irrigation water and soil solution are limiting factors that reduce plant growth and production (Hussain Khalid 2009). The salinity problem is increasing over time due to improper use of the canal irrigation system and also due to irrigation by salty groundwater. Leaf blight and defoliation are associated with the accumulation of toxic levels of  $\text{Na}^+$  and  $\text{Cl}^-$  in leaf cells. Changes in different physiological processes such as photosynthesis, enzymatic activity, stomatal conductivity, ion uptake, hormonal stability osmotic regulation, nucleic acid production, and protein synthesis are the main causes of reduced plant growth under salinity conditions. Decreased plant growth is also caused by an osmotic effect or a high accumulation of ions in plant tissues resulting in ion toxicity and nutritional imbalance (Patanè et al. 2013).

The main factors governing stress-induced conditions include plant species, cultivars, growth phases, soluble salt composition, duration and severity of stress, as well as natural climate conditions. Plant resistance to

several abiotic stresses, such as salt stress, is largely determined by various physiological and molecular mechanisms. The fruit plants contain a considerable number of metabolites that are identical to solutes and do not interfere with the plant's metabolic processes when subjected to salt stress. Salt-stressed plants often display an increase in proline. Proline is found to be more prevalent in fruit plants' stress resistance mechanisms. The salinity problem needs to be solved urgently for enhanced fruit production worldwide (Ben Abdallah et al. 2017). There can be several factors influencing the reduction of salt stress in fruit crops, including proteins, proteomics, molecular impacts, climatic fluctuations, and genetic variability. In fruit crops, the process of developing and identifying salt-tolerant varieties has been extremely slow. The importance of developing and identifying salt-tolerant cultivars becomes very evident under such conditions. Salt-tolerant cultivars are not likely to be developed by conventional approaches. To improve salt tolerance in salt-sensitive cultivars, however, it is essential to use techniques such as association mapping, quantitative trait loci (QTL) mapping, marker-assisted selection (MAS), and genome sequencing (Miranda-Apodaca et al. 2018).

The salt tolerance of annual crops and forages remains relatively constant throughout their life cycle, but the salt tolerance of fruit crops falls off after a few years. When fruit crops are exposed to high salinity stress in their initial years, salts are accumulated in their root and stem tissues. The accumulated salts, on the other hand, tend to move slowly toward the leaves due to long-term exposure to salinity. It is important to note that fruit crops are tolerant of excessive levels of salt during vegetative development but are adversely affected during reproductive development. Based on QTL information, interphase variability has been identified in salt tolerance mechanisms, so some species that will exhibit salt tolerance in the germination

stages may become salt sensitive in later phases of growth. As a fruit crop, salt toxicity has the most damaging effect and is harmful at reproductive stages, and partially edible or harvestable portions of the crop could also be affected (Ahmad and Anjum 2020).

Olive trees are more salt and drought tolerant than other temperate fruit cultivars, and they require less energy and nutrients than other fruit crops. At moderate concentrations, salinity affects pollen viability and germinability, the average proportion of complete blooms for each inflorescence, and fruit set but does not affect the size, fresh weight, or fruit drop. Salt tolerance regulatory components are encoded by the SOS1, SOS2, and SOS3 genes. It has been proposed that K plays an important role in salt tolerance. (Ozturk et al., 2021). Furthermore, salinity affects cellular and molecular reactions as well (Hussain et al., 2021).

### **1.3.1. Gene expression and salinity**

A variety of genes and signaling pathways are strongly activated in response to abiotic stress, allowing agricultural plants to adapt to the stress levels. The majority of regulation occurs at the transcriptional, post-transcriptional, and post-translational stages, with the transcriptional level receiving the most attention, which involves chromatin modification and overexpression and decreased expression of the gene's coding sequences (Lang et al., 2017). Salinity tolerance is a complex and ongoing genetic process controlled by several genes. Using genomic and transcriptome techniques, several transcription factors and salt-responsive genetic mutations have been identified. The SOS gene family is important in ion homeostasis in response to salinity (Sairam et al., 2004).

Transcription factors are considered primary regulators because they play an important role in determining gene expression. The transcription factor class including NAC, WRKY, C2H2, Bzip, DREB and APETALA2

(AP2), contains several stress-responsive genes. Scientists have discovered bZIP gene expression demonstrating overexpression of genes in salt-sensitive wheat varieties, but reduced expression of genes in salt-tolerant wheat varieties (Arabbeigi et al., 2019). The NAC transcription factor group causes gene upregulation in wheat and rice crops, resulting in salt tolerance and thus playing an important role in stress relief. Some transcription factors are regulated by various kinases, which play an important role in plant salt stress response. The transcription factor OsRMC in rice crops has been discovered to code for receptor-like kinases and is thought to be a promoter of the salinity stress response. It has also been demonstrated that salt, extreme cold, or ABA have no effect on the transcription level of the gene OsERBP1 but are slightly influenced by mild cold and droughts (Singh and colleagues, 2021).

#### **1.4. Flooding stress**

Moisture condenses the pores of waterlogged or swamped soils with inadequate drainage, preventing O<sub>2</sub> diffusion. When temperatures rise beyond 200 degrees Celsius, oxygen uptake by the root system, soil fauna, and soil microorganisms can completely deplete the oxygen from most of the soil moisture within 24 hours. As a result, anaerobic conditions develop, and the development, viability and yield of several species of plants are greatly diminished. TCA cannot function without O<sub>2</sub>, and ATP can only be synthesized by fermentation. Through all the activities of Lactate Dehydrogenase, roots start to convert pyruvate to lactate. Lactate fermentation is transitory because lactic acid production reduces cellular pH. Lactate Dehydrogenase is inhibited at acidic pH, but Pyruvate Decarboxylase is stimulated, resulting in the synthesis of ethanol via lactic acid (Nishiuchi et al., 2012)

#### **1.4.1. Responses of fruit crops to flooding and its management**

The capacity of plants to survive flooded soil varies greatly. Flooding causes changes to the soil that is often detrimental to the establishment and growth of agriculture. Fruit crops react to flooding in a way that is directly linked to the significant changes in oxygen supply and the physicochemical states of the land that follow floods. These alterations have a significant impact on growth and maturation. A variety of variables can impact plant tolerance to moist soil conditions, including soil type, chemistry, anaerobiosis degree, pathogens status, low vapor pressure, root and air temperature and developmental stages. The relevance of all of these elements varies depending on the plant species. It is the root system, not the scion which determines fruit tree resistance to flooded conditions, while particular foliar indications of flooding harm may fluctuate with scion (Ward, et al., 2020).

#### **1.5. Heat stress**

At higher temperatures, both photosynthesis and respiration are reduced, although photosynthetic rates decline before the respiration. The temperature compensation point refers to the temperature where a certain amount of CO<sub>2</sub> is emitted by respiration in each period. Photosynthesis cannot compensate for CO<sub>2</sub> utilized as a respiratory substrate above this temperature. As a result, carbohydrate resources are depleted, and fruits lack their taste. One of the primary causes of the negative consequences of high temperatures is an imbalance between photosynthesis and respiratory activities. The durability of a variety of cellular membranes is critical during heat stress, as well as chilling and freezing conditions. Extreme fluid in lipids at elevated heat is associated with physiological impairment. At extreme heat, the intensity of hydroxyl group and electrostatic forces among polar groups as well as the membrane's liquid phase deteriorate. As a result, integral proteins (that communicate both with hydrophilic and hydrophobic regions of the

membranes) tend to be more firmly associated with the lipid phase. High temperatures alter the composition and structure of the membrane, resulting in ion loss (Verma et al., 2020).

### **1.5.1. Response of fruit crops to heat stress and its management**

Sun exposure impairment in the form of huge black patches on the fruit pericarp can result from prolonged exposure to direct sunlight, rendering the fruit unsuitable. Fruits with an apical fruiting habit are more susceptible to sunlight. Several techniques might be implemented to minimize the frequency of sunburn. Different plant varieties may have greater leaf surfaces, giving better protection to fruits, and might have more resilient to sunburn. A better nutrition and irrigation program might boost vegetative development and thus shield the fruits against direct sunshine. Shadows or screens might also be created to protect plants and fruits from direct sunshine. An alternate method of preventing sunburn on fruit is to employ aerial watering to cool the canopy through evaporation (Araújo et al., 2022). This possibility is seldom in desert conditions because of water shortages and high salinity, which might harm the canopy due to salt stress. Studies are indicating that salicylic acid has a key role in plant response to biotic and abiotic stress. There have been several reports on the use of salicylic acid to improve tolerance against heat in fruits, vegetables, and ornamental plant species. Possible pathways of salicylic acid action in plant heat stress adaptations were also addressed (Liu et al., 2022). Although traditional methods such as sequencing techniques, QTL, MARS and MABC, are valuable in mapping, genetic variation studies and determination of genes linked to heat stress, they restrict the area in terms of complexity related to physiology, genetics, and molecular pathways. High throughput sequencing techniques and proteomics investigations provide an in-depth understanding of the process and concepts. CRISPR/Cas

9, transgenic studies and genetic engineering are advanced technologies that can help to reduce heat stress by manipulating agricultural plants to produce better heat tolerance. However, high throughput sequencing techniques are more expensive than traditional methods, thus out of reach for most farmers than traditional technology. Integration of both traditional and advanced methods can therefore be more effective and accessible. High throughput approaches indicate the significance and processes of exogenous application of many osmoprotectants and other proteins *in vivo*, which may be entrapped into the plant from external sources for sustainable agricultural development towards heat tolerance. There is currently a lot of research being done on heat stress, yet, additional attention is required to gain an understanding of the phenomena of heat stress. Modern technology should be employed to help farmers improve heat tolerance in agricultural species (Verma et al., 2020).

## **2. GENETIC IMPROVEMENT OF MAIN FRUIT THROUGH BIOTECHNOLOGICAL TOOLS**

Although genetically modified organisms (GMOs), particularly genetically modified plants (GMPs), are a valuable subject and the topic of intense debate, the general public is still very unfamiliar with them. Both acronyms are commonly used to refer to plants created using recombinant DNA technology, but they should be called Genetically Engineered Plants (GEPs). GEPs are the consequence of modifications and selection procedures based on current needs produced by humans over decades using any breeding strategy. GEPs, on the other hand, are produced by genome alterations induced by the insertion of genes extracted from any existing organism and transmitted via chemical, physical or biological (bacteria and viruses) vectors. These plants are currently referred to as "transgenics," since they involve the transcription of transgenes or the silencing of an indigenous protein to change a function. Genes/DNA can be transformed in species, however, the source of

the genes employed for the transformation is still unknown. This method has provoked vigorous debate among societies that are concerned with the transfer of genetic material across genotypes that would not naturally interbreed. Considering these criticisms, the cis-genic and intragenic ideas were proposed around a decade ago to differentiate modified plants based on the origin of advanced breeding genes or the alterations induced by the genetic variants of the plant exposed to alterations. As a result, such plants were termed "cis-genic" when plant species were genetically engineered with one or many genes isolated out of a crossable donor plant (including introns and surrounding elements including native promoters as well as terminator sequences in a core component). On the contrary, the approach "intragenic" refers to changes produced in a single gene by in vitro reorganization of different genes, i.e. they might contain genetic material from various genes and loci, allowing gene expression to be altered by employing various promoters or terminator sequences (Schouten *et al.*, 2006). From a legal perspective, both forms of plants are now recognized the very same. These two forms nevertheless have a similar phenomenon in which a unique sequence is inserted as randomly in the genome or the selected gene, as well as bacterial and viral noncoding vector regions e.g T-DNA borders (Zhuang *et al.*, 2014). It has been well understood that the expected total agricultural yield of cultivated plants worldwide is approximately 40% of total potential agricultural production, with shortfalls contributed to diseases (10-20%), drought stress (25%) bindweed (5-10%), cold exposure (16%), lack of oxygen (16%), salt concentrations pressure (5%) heavy metals (3%) and poor soils (20%). The present need for food is interpreted to explain global profits, beyond wasting and inadequate circulation, which cannot be met with simple production of virgin fertile lands, which is insufficient. However, with the involvement of those lands that are now unsuitable due to a lack of appropriate

plants, and typical genetic improvement procedures do not appear to be highly beneficial for such goals. Biotechnology works to meet this demand by developing plant varieties with a higher yield, quality, safety, and stability, to decrease pre and post-harvest waste in a short period. Furthermore, these techniques enable plant modification to make them suitable for "Phyto assisted remediation" or "bio-assisted phytoremediation," i.e. the advancement of plants with extensive and compact root systems to provide a high surface area accumulation and an enlarged rhizosphere, which together would form the root exudates a microhabitat beneficial to the microbes responsible for the degradation of organic pollutants in soils. This modification can be applied to plants that are already known for their proclivity to accumulate harmful metals to increase their accumulation capacity. However, for many plant species, technical barriers exist, such as barriers to genetic exchange in selection procedures and ineffective techniques of *in vitro* cultivation of plants from cells or tissues, mainly those of somatic origin. Despite these challenges, there has been a tremendous success, particularly with herbaceous plants, that have a short life span and are tiny enough to be tested *in vivo* in small places. This method, on the other hand, is best suited for plant species with a prolonged juvenile period, a high amount of heterozygosity, or little genetic diversity. In comparison to any other known technique, it provides for fast and accurate elimination of defects in commercialized cultivars/rootstocks without affecting or altering their essential qualities. In comparison to any other known technique, it allows for the rapid and precise removal of deficiencies in commercialized cultivars/rootstocks without affecting or changing their essential qualities. Contrary to popular opinion, these approaches have the potential to save endangered species while also improving the quality of our products. Minor "gene therapy" operations to correct the most serious flaws enable us to

preserve biodiversity, particularly cultivated types. The genetic improvement approaches and the most promising genes for fruit crops, as well as the most interesting results, are described in Table 2 (Rugini et al., 2020).

**Table 2.** Genetic transformation in fruit trees through genome editing tools.

<b>Fruit Cultivar</b>	<b>Genes</b>	<b>Results</b>	<b>References</b>
Apple	<i>MdbHLH104</i> <i>MdSLMYB1</i>	Tolerance against iron deficiency and Multiple abiotic stress.	(Wang et al., 2013)
Banana	<i>CBF1</i>	Resistance to cold hardiness	(Hu et al., 2013)
Citrus	Prolin synthesis	Drought Resistance	(Molinari et al., 2004)
Peach	<i>MbDREB1</i>	Cold, salinity and drought tolerance	(Yang et al., 2011)
Olive	<i>Osmotin</i>	Cold and salinity tolerance	(Rugini et al, 2016)
Kiwifruit	<i>AtNHX1</i>	Salinity tolerance	(Tian et al.,2011)
Wild grapevine	<i>VaCPK20</i>	Cold and drought tolerance	(Zhoa et al., 2016)
Plum	<i>Cytapx, cytsod</i>	Salt tolerance	Vivancos et al., 2013
Rough lemon	<i>Yeast (HAL2)</i>	Salt tolerance	(Ali et al., 2012)

### 2.1. Application of genome editing in fruit crops

Plant genetic modification technologies such as Zinc-Finger Nucleases (ZFNs), Trans Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) linked with Cas proteins are expanding crop improvement and functional genomics research opportunities. Various editing methods, particularly the CRISPR/Cas9 system, have been employed effectively in fruit crops including banana, citrus, apple, pear, kiwifruit and grapes. Recent research has shown that genetic engineering techniques have a substantial impact on plant bioengineering in general, but also in fruit crops. As these tools enable the modulation of multiple genes without gene transfer, such plants could be termed non-

transgenic. Editing tools enable the development of improved crop species that could have been approved even now in countries where transgenic crops are prohibited. Besides, genome-editing advancements produce high-quality product lines that would be nearly impossible to develop using conventional breeding techniques (Hussain et al., 2018). ZFNs and TALENs have been used in model organisms such as *Arabidopsis* and tobacco plants, and later in other crops, but their use in fruit crops is limited (Zhang et al., 2010; Butler et al., 2016). CRISPR/Cas9 technology has been used to generate the majority of technologies for fruit crops such as citrus, kiwifruit, apple, pear and banana (Erpen-Dalla Corte et al., 2019). Even though CRISPR/Cas9 system has been used in various crops for application domains such as NHEJ-mediated gene knockout, HDR-mediated gene replacement, gene targeting and rearranging, germline engineering, selection of a single trait, plant domestication, manufacturing of biopharmaceuticals, etc, research practices in fruit crops are still limited (Kaul et al., 2020). However, multiple studies were conducted to adapt the CRISPR/Cas9 technology for fruit cultivars (Ahmar et al., 2020), and various physiological pathways were investigated. Chlorophyll and carotenoid synthesis (Qin et al., 2007), juvenile phase and blooming phase (Varkonyi-Gasic et al., 2019), fruit development, and pest and disease response were all taken into account (Parkhi et al., 2018).

## **2.1. Genetic Engineering techniques in fruits**

Various biotechnological approaches can be employed to plants in terms of improving them throughout the plant breeding process. Tissue culture in crop species has been one of the most widely exploited areas of biotechnology. Tissue culture applications now reach well beyond rapid multiplication and micropropagation (Idowu et al., 2009). Another aspect of plant biotechnology is molecular marker technology. Molecular markers seem to be well known as useful plant breeding tools, and they have evolved into a

variety of systems based on various polymorphism-detecting techniques or approaches, such as SSR, RFLP, AFLP, RAPD, ISSR, SRAP, SNP, SSCP, etc (Jiang et al., 2013). Genetic engineering is a strong method for plant development, with the ability to integrate desirable traits into current genomes. Transformation technologies opened the way for key genes to be transferred into plant genomes to increase resistance to fungal, viral diseases, various pests, drought, and salt, as well as silencing unwanted genes and improving nutrient uptake (Mallikarjuna et al., 2016). GM technology offers both benefits and flaws. It can reduce the number of backcross generations by using to speed up the process of cleaning up the genetic background without changing the genetic makeup (no off-target effects). The requirement to know and sequence the target gene is a significant drawback of GM breeding. Other drawbacks of GM breeding include the need for specialist labs and the high cost, while less expensive, simpler alternatives are being explored (Forster et al., 2015). Table 3 illustrates several recent cases of genetic engineering techniques being used to transfer genes for fruits.

**Table 3.** Genetic engineering techniques used for gene transfer in various fruits.

<b>Plant species</b>	<b>Targeted gene</b>	<b>Transformation method</b>	<b>References</b>
<i>Citrus sinensis</i>	<i>Gfp, nptll, CsPDS</i>	Biolistic, Cas 9/sg RNA	(Wang at al., 2014)
<i>Vitis vinifera</i>	<i>VpPUB23</i>	Agrobacteriu tumifaciens.	(Mo et al., 2015)
<i>Malus domestica and ficus carica</i>	<i>uidA</i>	ZFN	(Peer et al., 2015)
<i>Prunus mume</i>	<i>Sgf(S65T)</i>	SAAT	(Gao-Takai, and Tao., 2014)
<i>C.paradisi</i>	<i>attE</i>	Agrobacteriu tumifaciens.	(Mondal et al., 2012)
<i>Vitis amurensis</i>	<i>RolB and nptll</i>	Agrobacteriu tumifaciens.	(Kiselev et al., 2007)
<i>Olea europaea</i>	<i>gus and nptll</i>	Biolistic	(Narvaez et al., 2018)

<i>Malus micromalus</i>	<i>RolC, gus</i>	Agrobacteriu tumifaciens	(Zhang et al., 2006)
<i>Coffea canephora</i>	<i>hptII, rol, uidA</i>	Agrobacteriuenesm rhizog	(Kumar et al., 2006)

Many fruit and vegetable crops, including the garden strawberry (*Fragaria X ananassa*), apple (*Malus domestica*), sweet orange (*Citrus sinensis*), tomato (*Solanum lycopersicum*), and squash, are produced through hybridization and selection (*Cucurbita maxima*). By using diploid and tetraploid ancestors, hybridization breeding may also be used to develop horticultural crops without seeds, such as watermelons. Crop hybridization breeding does, however, have constraints that are challenging to overcome. Although genetic engineering approaches and accelerated breeding procedures can speed up the breeding and selection processes yet needs tremendous quantities of labor and land resources. Modern genetic engineering techniques have recently begun to be used in addition to traditional gene transfer technologies for a wide range of plant species (Dönmez et al., 2016).

### 3. PERSPECTIVES AND CONCLUSIONS

From cereal production to diversified farming, the agricultural paradigm is already shifting. Aside from improving biological productivity and nutritional standards, horticultural crops can also significantly improve profitability, particularly fruit crops, which would constitute the core of any such agro-economic strategy for sustainable agriculture. To achieve enhanced production, cultivars and production technologies have been developed by studying the physiology of the growth and development process. With the changing environmental scenarios, many new issues, including climate change, have emerged as major challenges. To manage the stresses caused by temperature, light, and moisture regimes, we will need to develop a better understanding of both conventional management and molecular tools to cope

with these stresses. Dedicated efforts will be required to understand the physiological processes under various stresses. Climate change poses a threat to plant growth because of stress conditions, which will require an accelerated understanding of plant physiological processes and their molecular systems. Sustainable agriculture would be possible with committed biotechnological approaches combined with the physiology of fruit crops. These novel biotech approaches deal mainly with abiotic stress tolerance and ways to install tolerance mechanisms in fruit crops to achieve desired yields even in harsh environments. Molecular basis for plant heat tolerance and response to heat stress can be better understood through the application of genomics, proteomics, and transcriptomics approaches. An increasing number of molecules, their new roles, new concepts, and new mechanisms are being discovered in the field of abiotic stress tolerance. In addition, signal transduction pathways are becoming increasingly important, microarray analyses are becoming more common, and the field of functional genomics is becoming increasingly important.

#### 4. REFERENCES

- Abouzaid, E., El-Sayed, E. S. N., Mohamed, E. S. A., & Youssef, M. (2016). Molecular Analysis of Drought Tolerance in Guava based on *In Vitro* PEG Evaluation, *Tropical Plant Biology*, Vol. 9, No. 2, pp. 73-81.
- Aghdam, M. S., Sevillano, L., Flores, F. B., & Bodbodak, S. (2015). The Contribution of Biotechnology to Improving Post-Harvest Chilling Tolerance in Fruits and Vegetables Using Heat-Shock Proteins. *The Journal of Agricultural Science*, Vol. 153, No. 1, pp. 7-24.
- Ahmad, H. M., Rahman, M. U., Azeem, F., Tahir, N., Iqbal, & Mu, S. (2018). QTL Mapping for Crop Improvement Against Abiotic Stresses in Cereals. *Journal of Animal and Plant Sciences*, Vol. 28, pp. 1558–1573.
- Ahmad, H. M., Rahman, M. U., Farrukh, A., & Qurban, A. (2015). QTL Mapping for The Improvement of Drought Tolerance in Cereal Crops: An overview. *Life Science Journal*, Vol. 12, pp. 102–108.
- Ahmad, R., & Anjum, M. A. (2020). Physiological and Molecular Basis of Salinity Tolerance in Fruit Crops. *Fruit Crop Diagnosis Manag Nutr Constraints*, pp. 445–464. <https://doi.org/10.1016/B978-0-12-818732-6.00032-0>
- Ahmad, H. M., Wang, X., Rahman, M. U., Fiaz, S., Azeem, F., & Shaheen, T. (2021). Morphological and Physiological Response of *Helianthus annuus* L. to Drought Stress and Correlation of Wax Contents for Drought Tolerance Traits. *Arabian Journal for Science and Engineering*. DOI: 10.1007/s13369-021-06098-1.
- Ahmar, S., Saeed, S., Khan, M. H. U., Ullah Khan, S., Mora-Poblete, F., Kamran, M., ... & Jung, K. H. (2020). A Revolution Toward Gene-Editing Technology and Its Application to Crop Improvement. *International Journal of Molecular Sciences*, Vol. 21, No. 16, pp. 5665.
- Ahn, Y. J., & Song, N. H. (2012). A Cytosolic Heat Shock Protein Expressed in Carrot (*Daucus carota* L.) Enhances Cell Viability Under Oxidative and Osmotic Stress Conditions. *HortScience*, Vol. 47, No. 1, 143-148.
- Al-Abdallat, A. M., Al-Debei, H. S., Ayad, J. Y., & Hasan, S. (2014). Over-Expression of SISHN1 Gene Improves Drought Tolerance by Increasing Cuticular Wax Accumulation in Tomato. *International Journal of Molecular Sciences*, Vol. 15, pp. 19499–19515. DOI: 10.3390/ijms151119499.
- Alleva K., Marquez, M., Villarreal, N., Mut, P., Bustamante, C., Bellati, J., Martínez, G., Civello, M., & Amodeo, G. (2010). Cloning, Functional Characterization, and Co-Expression Studies of a Novel Aquaporin (FaPIP2;1) of Strawberry Fruit. *Journal of Experimental Botany*, Vol. 61, pp. 3935–3945. DOI: 10.1093/jxb/erq210.
- Ali, S., Mannan, A., El Oirdi, M., Waheed, A., & Mirza, B. (2012). *Agrobacterium*-Mediated Transformation of Rough Lemon (*Citrus jambhiri* Lush) with Yeast HAL2 Gene. *BMC Research Notes*, Vol. 5, No. 1, pp. 1-8.
- Arabbeigi, M., Arzani, A., & Majidi, M. M. (2019). Expression Profiles of P5CS and DREB2 Genes Under Salt Stress in *Aegilops cylindrica*. *Russian Journal of Plant Physiology*, Vol. 66, No. 4, pp. 583-590.

- Araújo, H. S. D., Carmo, S. A. D., Santos, N. C. B. D., Freitas, P. G. N., & Purquerio, L. F. V. (2022). Effect of Shading Screens on the Production and Quality of 'Smooth Cayenne' Pineapple. *Pesquisa Agropecuária Tropical*, Vol. 51.
- Bakshi, P., Jasrotia, A., & Wali, V. K. (2022). 10 Mitigation Strategies of Abiotic Stress in Fruit Crops. *Abiotic & Biotic Stress Management in Plants: Vol. I: Abiotic Stress*.
- Ben Abdallah, M., Trupiano, D., Ben Youssef, N., & Stefania Scippa, G. (2017). An Efficient Method for Olive Leaves Proteins Extraction and Two-Dimensional Electrophoresis. *The Natural Products Journal*, Vol. 7, No. 1, pp. 12-17.
- Biradar, J. (2013). Molecular Characterisation of Root Specific Mapping Population of Mulberry by SSR Markers and Identification of QTLs Governing Drought Tolerance Traits (Doctoral dissertation, University of Agricultural Sciences, GKVK).
- Butler, N. M., Baltes, N. J., Voytas, D. F., & Douches, D. S. (2016). Geminivirus-Mediated Genome Editing in Potato (*Solanum tuberosum* L.) Using Sequence-Specific Nucleases. *Frontiers in Plant Science*, Vol. 7, pp. 1045.
- Diao, D., Hu, X., Guan, D., Wang, W., Yang, H., & Liu, Y. (2020). Genome-Wide Identification of The ARF (auxin response factor) Gene Family in Peach and Their Expression Analysis. *Molecular Biology Reports*, Vol. 47, pp. 4331–4344. DOI: 10.1007/s11033-020-05525-0.
- Dönmez, D., Şimşek, Ö., & Kaçar, Y. A. (2016). Genetic Engineering Techniques in Fruit Science. *International Journal of Environmental and Agriculture Research*, Vol. 2, No. 12, pp. 115-128.
- Duan, M., Feng, H. L., Wang, L. Y., Li, D., & Meng, Q. W. (2012). Overexpression of Thylakoidal Ascorbate Peroxidase Shows Enhanced Resistance to Chilling Stress in Tomato. *Journal of Plant Physiology*, Vol. 169, No. 9, pp. 867-877.
- Erpen-Dalla Corte, L., M. Mahmoud, L., S. Moraes, T., Mou, Z., W. Grosser, J., & Dutt, M. (2019). Development of Improved Fruit, Vegetable, and Ornamental Crops Using The CRISPR/Cas9 Genome Editing Technique. *Plants*, Vol. 8, No. 12, pp. 601.
- Fan, Q. J., Yan, F. X., Qiao, G., Zhang, B. X., & Wen, X. P. (2014). Identification of Differentially-Expressed Genes Potentially Implicated in Drought Response in Pitaya (*Hylocereus undatus*) by Suppression Subtractive Hybridization and cDNA Microarray Analysis. *Gene*, Vol. 533, No. 1, pp. 322-331.
- Fernandes, R. D. M., Cuevas, M. V., Diaz-Espejo, A., & Hernandez-Santana, V. (2018). Effects of Water Stress on Fruit Growth and Water Relations Between Fruits and Leaves in A Hedgerow Olive Orchard, *Agricultural Water Management*, Vol. 210, pp. 32-40.
- Forster, B. P., Till, B. J., Ghanim, A. M. A., Huynh, H. O. A., Burstmayr, H., & Caligari, P. D. S. (2014). Accelerated Plant Breeding, *CABI Reviews*, Vol. 9, pp. 1–16.
- Fu, J., Wu, H., Ma, S., Xiang, D., Liu, R., & Xiong, L. (2017). OsJAZ1 Attenuates Drought Resistance by Regulating JA and ABA Signaling in Rice, *Frontiers in Plant Science*, Vol. 8. DOI: 10.3389/fpls.2017.02108.
- Gamboa-Tuz, S. D., Pereira-Santana, A., Zamora-Briseño, J. A., Castano, E., Espadas-Gil, F., Ayala-Sumuano, J. T., ... & Rodríguez-Zapata, L. C. (2018). Transcriptomics and Co-Expression Networks Reveal Tissue-Specific

- Responses and Regulatory Hubs Under Mild and Severe Drought in Papaya (*Carica papaya* L.). Scientific Reports, Vol. 8, No. 1, pp. 1-16.
- Gao-Takai, M., & Tao, R. (2013). Improving Infection Efficiency of *Agrobacterium* to Immature Cotyledon Explants of Japanese Apricot (*Prunus mume*) by Sonication Treatment. Journal of the Japanese Society for Horticultural Science, CH-085.
- Girard, A. L., Mounet, F., Lemaire-Chamley, M., Gaillard, C., Elmorjani, K., Vivancos, J., Runavot, J. L., Quemener, B., Petit, J., Germain, V., Rothan, C., Marion, D., & Bakan, B. (2012). Tomato GDSL1 Is Required for Cutin Deposition in the Fruit Cuticle, The Plant Cell, Vol. 24, pp. 3119–3134. DOI: 10.1105/tpc.112.101055.
- Hossain, A., Skalicky, M., Brestic, M., Maitra, S., Ashraf Al Alam, M., Syed, M. A., ... & Islam, T. (2021). Consequences and Mitigation Strategies of Abiotic Stresses in Wheat (*Triticum aestivum* L.) Under The Changing Climate. Agronomy, Vol. 11, No. 2, pp. 241.
- Hussain, K. (2009). Effect of Different Levels of Salinity on Growth and Ion Contents of Black Seeds (*Nigella sativa* L.), Current Research Journal of Biological Sciences, Vol. 1, No. 3, pp. 135-138.
- Hu, W., Hou, X., Huang, C., Yan, Y., Tie, W., Ding, Z., Wei, Y., Liu, J., Miao, H., Lu, Z., Li, M., Xu, B., & Jin, Z. (2015). Genome-Wide Identification and Expression Analyses of Aquaporin Gene Family during Development and Abiotic Stress in Banana, International Journal of Molecular Sciences, Vol. 16, pp. 19728–19751. DOI: 10.3390/ijms160819728.
- Hu, C., Liu, K., Wei, Y., Deng, G., Li, C., Kuang, R., ... & Yi, G. (2016). Overexpression of Arabidopsis CBF1 Gene in Transgenic Furenzhi Banana (*Musa* spp. AA group) Improves Resistance to Low Temperature, Molecular Plant Breeding, Vol. 7.
- Huang, D., Feurtado, J. A., Smith, M. A., Flatman, L. K., Koh, C., & Cutler, A. J. (2017). Long Noncoding miRNA Gene Represses Wheat  $\beta$ -Diketone Waxes, Proceedings of the National Academy of Sciences, Vol. 114, E3149–E3158. DOI: 10.1073/pnas.1617483114.
- Hussain, B., Lucas, S. J., & Budak, H. (2018). CRISPR/Cas9 in Plants: At Play in The Genome and at Work for Crop Improvement. Briefings in Functional Genomics, Vol. 17, No. 5, pp. 319-328.
- Hussain, S., Hussain, S., Ali, B., Ren, X., Chen, X., Li, Q., ... & Ahmad, N. (2021). Recent Progress in Understanding Salinity Tolerance in Plants: Story of Na<sup>+</sup>/K<sup>+</sup> Balance and Beyond, Plant Physiology and Biochemistry, Vol. 160, pp. 239-256.
- Idowu, P. E., Ibitoye, D. O., & Ademoyegun, O. T. (2009). Tissue Culture as A Plant Production Technique for Horticultural Crops. African Journal of Biotechnology, Vol. 8, No. 16.
- Ijaz, M., Shahzadi, R., Masoud, M. S., Iqbal, M., & Umirbekovna, I. A. (2020). Transcription Factors and Plant Abiotic Stress Responses. In: Hasanuzzaman M (ed) Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives 1, 1st edn. Springer, Singapore, pp 663–681.

- Ijaz, M., Qamar, S., Bukhari, S. A., & Malik, K. (2019). Abiotic Stress Signaling in Rice Crop, In *Advances in Rice Research for Abiotic Stress Tolerance*, pp. 551-569. Woodhead Publishing.
- Jiang, G. L. (2013). Molecular Markers and Marker-Assisted Breeding in Plants, *Plant Breeding from Laboratories to Fields*, Vol. 3, pp. 45-83.
- Kaul, T., Sony, S. K., Verma, R., Motelb, K. F. A., Prakash, A. T., Eswaran, M., ... & Kaul, R. (2020). Revisiting CRISPR/Cas-Mediated Crop Improvement: Special Focus on Nutrition. *Journal of Biosciences*, Vol. 45, No. 1, pp. 1-37.
- Khanum, Z., Tiznado-Hernández, M. E., Ali, A., Musharraf, S. G., Shakeel, M., & Khan, I. A. (2020). Adaptation Mechanism of Mango Fruit (*Mangifera indica* L. cv. Chaunsa White) to Heat Suggest Modulation in Several Metabolic Pathways, *RSC Advances*, Vol. 10, No. 58, pp. 35531-35544.
- Kiselev, K. V., Dubrovina, A. S., Veselova, M. V., Bulgakov, V. P., Fedoreyev, S. A., & Zhuravlev, Y. N. (2007). The rolB Gene-Induced Overproduction of Resveratrol in *Vitis amurensis* Transformed Cells, *Journal of Biotechnology*, Vol. 128, No. 3, pp. 681-692.
- Kumar, A., Sreedharan, S. P., Shetty, N. P., & Parvatam, G. (2016). Developing Sustainable Disease Resistance in Coffee: Breeding vs. Transgenic Approaches, *Plant Pathogen Resistance Biotechnology*, Vol. 217, pp. 43.
- Lang, L., Xu, A., Ding, J., Zhang, Y., Zhao, N., Tian, Z., ... & Huang, Z. (2017). Quantitative Trait Locus Mapping of Salt Tolerance and Identification of Salt-Tolerant Genes in *Brassica napus* L, *Frontiers in Plant Science*, Vol. 8, pp. 1000.
- Lashbrooke, J., Aharoni, A., & Costa, F. (2015). Genome Investigation Suggests MdSHN3 an APETALA2-Domain Transcription Factor Gene, to Be A Positive Regulator of Apple Fruit Cuticle Formation and an Inhibitor of Russet Development, *Journal of Experimental Botany*, Vol. 66, pp. 6579–6589. DOI: 10.1093/jxb/erv366.
- Leide, J., Hildebrandt, U., Reussing, K., Riederer, M., & Vogt, G. (2007). The Developmental Pattern of Tomato Fruit Wax Accumulation and Its Impact on Cuticular Transpiration Barrier Properties: Effects of a Deficiency in a beta-Ketoacyl-Coenzyme A Synthase (LeCER6), *Plant Physiology*, Vol. 144, pp. 1667–1679. DOI: 10.1104/pp.107.099481.
- Levy, Y., & Syvertsen, J. (2010). Irrigation Water Quality and Salinity, Effects in Citrus Trees. *Horticultural Reviews*, Vol. 30, pp. 37-82.
- Li, L., Luo, C., Huang, F., Liu, Z., An, Z., Dong, L., & He, X. (2019). Identification and Characterization of The Mango eIF Gene Family Reveals MielF1A-a, Which Confers Tolerance to Salt Stress in Transgenic Arabidopsis, *Scientia Horticulturae*, Vol. 248, pp. 274-281.
- Liu, J., Qiu, G., Liu, C., Li, H., Chen, X., Fu, Q., ... & Guo, B. (2022). Salicylic Acid, a Multifaceted Hormone, Combats Abiotic Stresses in Plants, *Life*, Vol. 12, No. 6, pp. 886.
- Liu, W., Cheng, C., Lin, Y., XuHan, X., & Lai, Z. (2018). Genome-Wide Identification and Characterization of mRNAs and lncRNAs Involved in Cold Stress in the Wild Banana (*Musa itinerans*), *PLoS One*, Vol. 13, No. 7, e0200002.

- López-Galiano, M. J., García-Robles, I., González-Hernández, A. I., Camañes, G., Vicedo, B., Real, M. D., & Rausell, C. (2019). Expression of miR159 Is Altered in Tomato Plants Undergoing Drought Stress, *Plants*, Vol. 8. DOI: 10.3390/plants8070201.
- Mace, E. S., Singh, V., Van Oosterom, E. J., Hammer, G. L., Hunt, C. H., & Jordan, D. R. (2012). QTL for Nodal Root Angle in Sorghum (*Sorghum bicolor* L. Moench) Co-Locate with QTL for Traits Associated with Drought Adaptation, *Theoretical and Applied Genetics*, Vol. 124, pp. 97–109. DOI: 10.1007/s00122-011-1690-9.
- Mallikarjuna, G., Rao, T. S. R. B., & Kirti, P. B. (2016). Genetic Engineering for Peanut Improvement: Current Status and Prospects. *Plant Cell, Tissue and Organ Culture*, Vol. 125, No. 3, pp. 399-416.
- Meena, K. K., Sorty, A. M., Bitla, U. M., Choudhary, K., Gupta, P., Pareek, A., ... & Minhas, P. S. (2017). Abiotic Stress Responses and Microbe-Mediated Mitigation in Plants: The Omics Strategies, *Frontiers in Plant Science*, Vol. 8, pp. 172.
- Miranda-Apodaca, J., Yoldi-Achalandabaso, A., Aguirresarobe, A., et al. (2018). Similarities and Differences Between The Responses to Osmotic and Ionic Stress in Quinoa from A Water Use Perspective. *Agricultural Water Management*, Vol. 203, pp. 344–352.
- Mo, R., Huang, Y., Yang, S., Zhang, Q., & Luo, Z. (2015). Development of *Agrobacterium*-Mediated Transient Transformation in Persimmon (*Diospyros kaki* Thunb.). *Scientia Horticulturae*, Vol. 192, pp. 29-37.
- Molinari, H. B. C., Marur, C. J., Bessalho Filho, J. C., Kobayashi, A. K., Pileggi, M., Júnior, R. P. L., ... & Vieira, L. G. E. (2004). Osmotic Adjustment in Transgenic Citrus Rootstock Carrizo Citrange (*Citrus sinensis* Osb. x *Poncirus trifoliata* L. Raf.) Overproducing Proline, *Plant Science*, Vol. 167, No. 6, pp. 1375-1381.
- Mondal, S. N., Dutt, M., Grosser, J. W., & Dewdney, M. M. (2012). Transgenic Citrus Expressing The Antimicrobial Gene Attacin E (*attE*) Reduces The Susceptibility of ‘Duncan’ Grapefruit to The Citrus Scab Caused by *Elsinoë fawcettii*, *European Journal of Plant Pathology*, Vol. 133, No. 2, pp. 391-404.
- Narvaez, I., Khayreddine, T., Pliego, C., Cerezo, S., Jiménez-Díaz, R. M., Trapero-Casas, J. L., ... & Pliego-Alfaro, F. (2018). Usage of The Heterologous Expression of The Antimicrobial Gene *afp* from *Aspergillus giganteus* for Increasing Fungal Resistance in Olive, *Frontiers in Plant Science*, Vol. 9, pp. 680.
- Negrão, S., Schmöckel, S. M., & Tester, M. (2017). Evaluating Physiological Responses of Plants to Salinity Stress. *Annals of Botany*, Vol. 119, No. 1, pp. 1-11.
- Nie, Q., Gao, G. L., Fan, Q. J., Qiao, G., Wen, X. P., Liu, T., ... & Cai, Y. Q. (2015). Isolation and Characterization of A Catalase Gene “HuCAT3” from Pitaya (*Hylocereus undatus*) and Its Expression Under Abiotic Stress, *Gene*, Vol. 563, No. 1, pp. 63-71.
- Nishiuchi, S., Yamauchi, T., Takahashi, H., Kotula, L., & Nakazono, M. (2012). Mechanisms for Coping with Submergence and Waterlogging in Rice, *Rice*, Vol. 5, No. 1, pp. 1-14.

- Ozturk, M., Altay, V., Gönenç, T. M., Unal, B. T., Efe, R., Akçiçek, E., & Bukhari, A. (2021). An Overview of Olive Cultivation in Turkey: Botanical Features, Eco-Physiology and Phytochemical Aspects, *Agronomy*, Vol. 11, No. 2, pp. 295.
- Parkhi, V., Bhattacharya, A., Choudhary, S., Pathak, R., Gawade, V., Palan, B., ... & Char, B. (2018). Demonstration of CRISPR-cas9-Mediated *pds* Gene Editing in A Tomato Hybrid Parental Line, *Indian Journal of Genetics and Plant Breeding*, Vol. 78, No. 01, pp. 132-137.
- Parmar, N., Singh, K. H., Sharma, D., Singh, L., Kumar, P., Nanjundan, J., ... & Thakur, A. K. (2017). Genetic Engineering Strategies for Biotic and Abiotic Stress Tolerance and Quality Enhancement in Horticultural Crops: A Comprehensive Review, *3 Biotech*, Vol. 7, No. 4, pp. 1-35.
- Patanè, C., Saita, A., & Sortino, O. (2013). Comparative Effects of Salt and Water Stress on Seed Germination and Early Embryo Growth in Two Cultivars of Sweet Sorghum, *Journal of Agronomy and Crop Science*, Vol. 199, pp. 30–37. <https://doi.org/10.1111/J.1439-037X.2012.00531.X>
- Peer, R., Rivlin, G., Golobovitch, S., Lapidot, M., Gal-On, A., Vainstein, A., ... & Flaishman, M. A. (2015). Targeted Mutagenesis Using Zinc-Finger Nucleases in Perennial Fruit Trees, *Planta*, Vol. 241, No. 4, pp. 941-951.
- Pieczynski, M., Marczewski, W., Hennig, J., Dolata, J., Bielewicz, D., Piontek, P., Wyrzykowska, A., Krusiewicz, D., Strzelczyk-Zyta, D., Konopka-Postupolska, D., Krzeslowska, M., Jarmolowski, A., & Szweykowska-Kulinska, Z. (2013). Down-Regulation of CBP80 Gene Expression as A Strategy to Engineer A Drought-Tolerant Potato, *Plant Biotechnology Journal*, Vol. 11, pp. 459–469. DOI: 10.1111/pbi.12032.
- Qi, C., Jiang, H., Zhao, X., Mao, K., Liu, H., Li, Y., & Hao, Y. (2019). The Characterization, Authentication, and Gene Expression Pattern of the MdCER Family in *Malus domestica*, *Horticultural Plant Journal*, Vol. 5, pp. 1–9. DOI: 10.1016/j.hpj.2018.11.003.
- Qin, G., Gu, H., Ma, L., Peng, Y., Deng, X. W., Chen, Z., & Qu, L. J. (2007). Disruption of Phytoene Desaturase Gene Results in Albino and Dwarf Phenotypes in Arabidopsis by Impairing Chlorophyll, Carotenoid, and Gibberellin Biosynthesis, *Cell Research*, Vol. 17, No. 5, pp. 471-482.
- Rahman, H., Pekic, S., Lazic-Jancic, V., Quarrie, S. A., Shah, S. M., Pervez, A., & Shah, M. M. (2011). Molecular Mapping of Quantitative Trait Loci for Drought Tolerance in Maize Plants, *Genetics and Molecular Research*, DOI: 10.4238/vol10-2gmr1139.
- Rugini, E., & Silvestri, C. (2016). Somatic Embryogenesis in Olive (*Olea europaea* L. subsp. *europaea* var. *sativa* and var. *sylvestris*), In *In Vitro Embryogenesis in Higher Plants*, pp. 341-349. Humana Press, New York, NY.
- Rugini, E., Bashir, M. A., Cristofori, V., Ruggiero, B., & Silvestri, C. (2020). A Review of Genetic Improvement of main Fruit Trees Through Modern Biotechnological Tools and Considerations of The Cultivation and Research of The Engineered Plant Restrictions, *Pakistan Journal of Agricultural Sciences*, Vol. 57, No. 1, pp. 17-42.
- Sairam, R. K., & Tyagi, A. (2004). Physiology and Molecular Biology of Salinity Stress Tolerance in Plants, *Current Science*, pp. 407-421.

- Salih, H., Tan, L., & Htet, N. N. W. (2021). Genome-Wide *identification, characterization* of bHLH Transcription Factors in Mango, *Tropical Plant Biology*, Vol. 14, No. 1, pp. 72-81.
- Schouten, H. J., Krens, F. A., & Jacobsen, E. (2006). Cisgenic Plants Are Similar to Traditionally Bred Plants: International Regulations for Genetically Modified Organisms Should Be Altered to Exempt Cisgenesis, *EMBO Reports*, Vol. 7, No. 8, pp. 750-753.
- Sharma, A., Ruiz-Manriquez, L. M., Serrano-Cano, F. I., Reyes-Pérez, P. R., Tovar Alfaro, C. K., Barrón Andrade, Y. E., ... & Paul, S. (2020). Identification of microRNAs and Their Expression in Leaf Tissues of Guava (*Psidium guajava* L.) Under Salinity Stress, *Agronomy*, Vol. 10, No. 12, pp. 1920.
- Singh, M., Nara, U., Kumar, A., Choudhary, A., Singh, H., & Thapa, S. (2021). Salinity Tolerance Mechanisms and Their Breeding Implications, *Journal of Genetic Engineering and Biotechnology*, Vol. 19, No. 1, pp. 1-18.
- Solankey, S. S., Singh, R. K., Baranwal, D. K., & Singh, D. K. (2015). Genetic Expression of Tomato for Heat and Drought Stress Tolerance: An Overview, *International Journal of Vegetable Science*, Vol. 21, pp. 496–515. DOI: 10.1080/19315260.2014.902414.
- Szakiel, A., Paćzkowski, C., Pensec, F., & Bertsch, C. (2012). Fruit Cuticular Waxes as A Source of Biologically Active Triterpenoids, *Phytochemistry Reviews*, DOI: 10.1007/s11101-012-9241-9.
- Tian, N., Wang, J., & Xu, Z. Q. (2011). Overexpression of Na<sup>+</sup>/H<sup>+</sup> Antiporter Gene AtNHX1 from *Arabidopsis thaliana* Improves The Salt Tolerance of Kiwifruit (*Actinidia deliciosa*), *South African Journal of Botany*, Vol. 77, No. 1, pp. 160-169.
- Varkonyi-Gasic, E., Wang, T., Voogd, C., Jeon, S., Drummond, R. S., Gleave, A. P., & Allan, A. C. (2019). Mutagenesis of Kiwifruit Centroradialis-like Genes Transforms A Climbing Woody Perennial with Long Juvenility and Axillary Flowering into A Compact Plant with Rapid Terminal Flowering, *Plant Biotechnology Journal*, Vol. 17, No. 5, pp. 869-880.
- Vasanthaiiah, H. K., Ravishankar, K. V., Narayanaswamy, P., & Shivashankara, K. S. (2008). Influence of Temperature on Spongy Tissue Formation in ‘Alphonso’ Mango, *International Journal of Fruit Science*, Vol. 8, No. 3, pp. 226-234.
- Wang, R. K., Cao, Z. H., & Hao, Y. J. (2014). Overexpression of A R2R3 MYB Gene MdSIMYB1 Increases Tolerance to Multiple Stresses in Transgenic Tobacco and Apples, *Physiologia Plantarum*, Vol. 150, No. 1, pp. 76-87.
- Wang, Y., Cai, S., Yin, L., Shi, K., Xia, X., Zhou, Y., Yu, J., & Zhou, J. (2015). Tomato HsfA1a Plays A Critical Role in Plant Drought Tolerance by Activating ATG Genes and Inducing Autophagy, *Autophagy*, Vol. 11, pp. 2033–2047.
- Wang, L., Li, Q. T., Lei, Q., Feng, C., Zheng, X., Zhou, F., Li, L., Liu, X., Wang, Z., & Kong, J. (2017). Ectopically Expressing MdPIP1;3, an Aquaporin Gene, Increased Fruit Size and Enhanced Drought Tolerance of Transgenic Tomatoes, *BMC Plant Biology*, Vol. 17, pp. 246. DOI: 10.1186/s12870-017-1212-2.1080/15548627.2015.1098798.

- Ward, P. J., de Ruiter, M. C., Mård, J., Schröter, K., Van Loon, A., Veldkamp, T., ... & Wens, M. (2020). The Need to Integrate Flood and Drought Disaster Risk Reduction Strategies, *Water Security*, Vol. 11.
- Wu, J., Cao, J., Su, M., Feng, G., Xu, Y., & Yi, H. (2019). Genome-Wide Comprehensive Analysis of Transcriptomes and Small RNAs Offers Insights into The Molecular Mechanism of Alkaline Stress Tolerance in A Citrus Rootstock, *Horticulture Research*, Vol. 6.
- Yang, W., Liu, X. D., Chi, X. J., Wu, C. A., Li, Y. Z., Song, L. L., ... & Li, H. Y. (2011). Dwarf Apple MbDREB1 Enhances Plant Tolerance to Low Temperature, Drought, and Salt Stress via Both ABA-Dependent and ABA-Independent Pathways, *Planta*, Vol. 233, No. 2, pp. 219-229.
- Yun, Z., Jin, S., Ding, Y., Wang, Z., Gao, H., Pan, Z., ... & Deng, X. (2012). Comparative Transcriptomics and Proteomics Analysis of Citrus Fruit, to Improve Understanding of The Effect of low Temperature On Maintaining Fruit Quality During Lengthy Post-Harvest Storage, *Journal of Experimental Botany*, Vol. 63, No. 8, pp. 2873-2893.
- Zhang, F., Maeder, M. L., Unger-Wallace, E., Hoshaw, J. P., Reyon, D., Christian, M., ... & Voytas, D. F. (2010). High Frequency Targeted Mutagenesis in *Arabidopsis thaliana* using Zinc Finger Nucleases, *Proceedings of the National Academy of Sciences*, Vol. 107, No. 26, pp. 12028-12033.
- Zhang, Z., Sun, A., Cong, Y., Sheng, B., Yao, Q., & Cheng, Z. M. (2006). *Agrobacterium*-mediated Transformation of The Apple Rootstock *Malus micromalus* Makino with The RolC Gene, *In Vitro Cellular & Developmental Biology-Plant*, Vol. 42, No. 6, pp. 491-497.
- Zhao, Q., Ren, Y. R., Wang, Q. J., Yao, Y. X., You, C. X., & Hao, Y. J. (2016). Overexpression of Mdb HLH 104 Gene Enhances The Tolerance to Iron Deficiency in Apple, *Plant Biotechnology Journal*, Vol. 14, No. 7, pp. 1633-1645.
- Zhou, J., Wang, Z., Mao, Y., Wang, L., Xiao, T., Hu, Y., ... & Ma, Y. (2020). Proteogenomic Analysis of Pitaya Reveals Cold Stress-Related Molecular Signature, *Peer Journal*, Vol. 8, e8540.
- Zhu, L. H., van de Peppel, A., Li, X. Y., & Welander, M. (2004). Changes of Leaf Water Potential and Endogenous Cytokinins in Young Apple Trees Treated with or without Paclobutrazol Under Drought Conditions, *Scientia Horticulturae*, Vol. 99, No. 2, pp. 133-141.
- Zhu, X., Luo, J., Li, Q., Li, J., Liu, T., Wang, R., ... & Li, X. (2018). Low Temperature Storage Reduces Aroma-Related Volatiles Production During Shelf-Life of Banana Fruit Mainly by Regulating Key Genes Involved in Volatile Biosynthetic Pathways, *Postharvest Biology and Technology*, Vol. 146, pp. 68-78.
- Zhuang, J., Zhang, J., Hou, X. L., Wang, F., & Xiong, A. S. (2014). Transcriptomic, Proteomic, Metabolomic and Functional Genomic Approaches for The Study of Abiotic Stress in Vegetable Crops, *Critical Reviews in Plant Sciences*, Vol. 33, No. 2-3, pp. 225-237.

## CHAPTER 8

### DIGITAL APPLICATIONS IN TURKISH AGRICULTURE

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## INTRODUCTION

Today, digitalization is of importance in term of businesses to compete with their competitors, to protect and develop their presence in the market. The pandemic process that we experienced has also caused to this digital world to get in our lives faster and to adapt to these digital applications. Today, digital applications are available in every sector and also every vary of business. Especially in the agricultural sector; Digital applications are to become more crucial thanks to the unique characteristics of the sector, the behavior of the producers, the structure and processing of agricultural enterprises, and its relationship with agricultural input and agro-industrial sectors. On the other hand, in the view of the fact that the critical role played by the agricultural sector in the development of the country, the sector should be strengthened with information and communication technologies. According to the datas, the agricultural gross domestic product value of our country is approximately 50 billion dollars and its share in the total GDP is 6.4%. It is stated that the share of the agricultural sector in employment is around 20%. Turkey, develops a number of policies so as to maximize this power, has in agriculture. Digitalization in agriculture is one of these policies. (Pakdemirli, Birişik, Aslan, Sönmez & Gezici, 2021).

Recently, concepts such as digitalization and digital transformation have been frequently encountered. The concept of digital comes from the Latin word “digitus” meaning finger and refers to digitization in information systems (Klein, 2020). Accordingly, digitalization is named that country, industry or organization etc. adopt to computer technologies and use these technologies in all processes. More with precise expression, in order to reduce costs and increase efficiency, it is the enhancement or improvement of the efficiency of all processes through digital technologies and digitized data. (Tutkunca & Haydar, 2022). On the other hand, the results of the studies show

that many businesses are striving to achieve transformative effects from new digital technologies, but a significant minority are able to develop their management and technology skills in applying the potential of new technologies. In Fact, businesses using digital technology effectively can take advantage of at least one or more of these three areas, such as better customer experience and loyalty, improved operations, new business lines or models (Fitzgerald, Kruschwitz, Bonnet & Welch, 2013). This is seen that the agricultural sector has also been affected by the digitalization process experienced in the industrial sector with the effect of Industry 4.0 in the recent period. With digitalization, smart tools have been ready used in agricultural enterprises. It is possible to facilitate agricultural activities by analyzing the weather and soil conditions, estimated harvest time, where, how much and what kind of fertilizer or pesticide should be used, irrigation processes, minerals needed by plants, or the fight against pests with these smart tools (Çakır & İşlek, 2021). By the end of the 18th century, the use of water and steam power in production is stated as Industry 1.0. With the prevalence use of electricity in production at the beginning of the 20th century, mass production was started and this period was called Industry 2.0. By the 1970s, the use of computers, electronics, information technologies and automation systems became widespread, leading to the Industry 3.0 period.

Today's Industry 4.0 is the name given to digital transformation, involving many areas such as fully autonomous systems, smart robots, pilotless aircraft, driverless cars, virtual reality (Kırkaya, 2019). At the core of Industry 4.0 are networked intelligent systems being able to regulate production and allow people, machines, equipment and products to communicate with each other (Guban & Kovacs, 2017). The purpose of using these practices in the agricultural sector is to maximize agricultural efficiency and productivity. In today's world, where agricultural areas are getting

smaller, water resources decreasing, risks related to food safety increasing, and the global climate crisis is affecting our world, the importance of digitalization in the agricultural sector emerges.

In this context, the aim of this study is to reveal the digital applications seen in Turkish agriculture. The limitation of the study is to examine the digital applications made by the Ministry of Agriculture and Forestry, the Ministry of Commerce, the finance sector, producer organizations and the private sector, having significant effects on Turkey's agriculture with the policies and projects they have developed, in line with this purpose.

## **2. 4.0 APPLICATION TOOLS IN AGRICULTURE AND DIGITAL APPLICATIONS IN AGRICULTURE WITH EXAMPLES FROM THE WORLD**

### **2.1. 4.0 Implementation Tools in Agriculture**

In the 1600s, changes in British agriculture in order to meet the increasing food demand due to population growth paved the way for the 1st Industrial Revolution and this period was called the Agricultural Revolution. While efficiency in agriculture increased with the consolidation of agricultural lands and the emergence of agricultural machinery in England, productivity increased even more with the use of fertilizers in the 1840s. With the 2nd Industrial Revolution between 1870-1914, electricity commence to be used, mass production was started, mechanization in agriculture increased, chemical fertilizers and pesticides for agricultural pests commence to be used. By the 1960s, the 3rd Industrial Revolution, called the computer and digital revolution, was entered. The changes to have emerged in the agricultural sector with the use of mechanization, fertilization, irrigation, pesticides and technological knowledge in agriculture are called the Green Revolution. Satellite technologies were commenced to be used in 1994 in order to monitor

the farms and make more accurate planning. In the 2000s, software and mobile devices became used in the agricultural sector. In 2011, the German Federal Government introduced this advanced technology strategy in agriculture with the concept of Agriculture 4.0. (Kılavuz & Erdem, 2019). In the literature, it is also seen that the concept of agriculture 4.0 is referred to with the concepts of smart agriculture or digital agriculture. (Pogorelskaia & Varallyai, 2020). Agriculture 4.0 capitalize on a number of information and communication technologies to increase productivity in agriculture, investigate the variability of agricultural data and cope with changes in the agricultural scenario (Sott et al., 2020). These tools are briefly described below.

**2.1.1. Internet of Things (IoT):** With the IoT technology placed in agricultural fields, producers are able to access the data of plants remotely. These devices consist of several sensors that examine several parameters in parallel, such as soil moisture content, temperature or soil electrical conductivity, affecting plant growth. In this way, plants are monitored in real time and in the light of the data obtained and determined for example, when irrigation, fertilization or pesticide use is needed (Pogorelskaia & Varallyai, 2020).

**2.1.2. Big Data:** Data collected from the field or farm includes information such as planting, agricultura spraying, necessary materials, yield, soil types, weather conditions and seasonal images (Wolfert, Ge, Verdouw & Bogaardt, 2017). Big data is an application that enables to make decision through the use of these data mentioned together. With this application, three important benefits such as cost reduction, improvement in decision making and improvement in products and services are provided (Özsoylu, 2017). Big data provides manufacturers with predictive innervation into their production

activities and real-time operational decisions and business process redesign (Zhai, Martinez, Beltran & Martinez, 2020).

**2.1.3. Use of Satellites and Drones:** Within the framework of precision agriculture practices, satellites and aircraft have been used to obtain information about agricultural areas for a long time. As a result of the developments in drone technology, the use of these vehicles for agricultural purposes has been widespread. It also enable to possible for producers to access the images they need instantly or to carry out activities such as pesticide applications remotely at low cost. Drones, custom engineered for agricultural applications have sensitive sensors and imaging systems to detect pests and plant diseases (Ozdogan, Gacar & Aktas, 2017).

**2.1.4. Robotic Systems and Autonomous Vehicles:** Farmer robots or autonomous agricultural machines are effectively used in agricultural activities. Autonomous tractors that precisely plow and seed the planting area or autonomous combine harvesters that separate the product and the stalk are examples of autonomous vehicles. Farmer robots, on the other hands, implement many tasks, from sowing the seed to the point where the highest yield will be obtained by analyzing the planting area, to detect the harmful weeds in the planting area and applying the most appropriate pesticide (Ercan, Öztepe, Güler & Saner, 2019).

**2.1.5. Cloud Computing System:** A cloud computing system is defined as a service structure that allows users' data to be accessed or run applications at any time via a server in the internet environment (Özsoylu, 2017). In other words, cloud computing is the providing of computer or information technology infrastructure over the internet. With the cloud computing system, all data related to soil, weather, crop and producer can be stored in one place and data availability to be ensured. Thusly, users such as

producers, researchers, consultants and experts can easily access this data from anywhere and at any time via devices connected to the cloud system (Choudhary, Jadoun & Mandoria, 2016).

**2.1.6. Image Processing Technology:** The basic definition of image processing is the processing of digital image, more precisely, the elimination of noise and all kinds of irregularities in the image by means of digital computers. It is used in many fields such as image processing, medical imaging, military, materials science, graphic arts, printing industry, textile, forensic research (Chitradevi & Srimathi, 2014) and it has been frequently used in agricultural activities in recent years. When agricultural activities are carried out using traditional methods, producers control their products by using their hands and eyes. However, when image processing methods are used for control purposes, possible to reach some numerical data over the obtained image and thus to reach new results. In this context, these systems are of importance in activities such as product quality control, product classification and automation (Kaymak, Örnek & Kahramanlı, 2019). In summary, these tools, to be applied at every stage of agricultural activities, have benefits such as providing quality and safe food, reducing the costs of chemical raw materials such as pesticides and fertilizers, creating an agricultural registration system, and accessing accurate information for more effective aquaculture and management decisions (Kaya, 2019).

## **2.2. Digital Applications in Agriculture with Examples from the World**

Some examples of agricultural 4.0 practices in the world are given below.

**2.2.1. England:** England realizes digital agriculture practices with the cooperation of universities, industry and government, and for this purpose, it stands out with the support given to education and the research centers established. Rothamsted Institute, one of the leading organizations in England, works on environmentally friendly agricultural technologies and carries out successful projects. Researchers at the institute provide information sharing by making hundreds of articles published every year accessible worldwide. The institution carries out genetic studies so as to ensure food safety and increase productivity. Moreover, in 2015, they established the first field crops analysis facility in the world. One of the most important collaborations on agriculture in the country is the N8 agriculture platform. Universities of Manchester, Leeds, Newcastle, York, Lanchester, Liverpool, Durham and Sheffield have come together on the N8 agriculture platform. In line with the objectives of the platform, agriculture 4.0 technologies and gene studies are focused on. Also, continues to work on plant and food health, safe food supply chain and sustainable food production (Saygılı, Kaya, Tunalı Çalışkan & Erdölek Kozal, 2019).

**2.2.2. Netherlands:** The Netherlands attaches importance to sustainable and technology-based agricultural policies. Renewable energy and high efficiency irrigation systems, robots, big data analysis, automation systems, smart software are used in the country. For example, the government purchased 1.4 million Euro worth of satellite data in order to increase productivity in agriculture, and based on this data, it provided information to its producers on issues such as atmosphere, soil and crop development (Kirmikil & Ertaş, 2020).

**2.2.3. Israel:** Israel is one of the important countries in the field of agriculture with its research and development investments in the agricultural

sector and mechanization. In the country where soil and water scarcity is experienced, remarkable achievements have been achieved by using advanced technologies. Biological pest control, computer-controlled drip irrigation, thermal imaging in crop water stress detection, drought-resistant seed varieties can be counted among the projects carried out. The drip irrigation method, considered the most important development in modern agriculture, was invented by Israel in 1959. Irrigation software called CropX has been synchronized to smart phones, thus paving the way for real-time data access on weather and soil conditions. In the greenhouses located in the agricultural center called Arava in the desert, fertilization and irrigation are carried out using computers. Pytech, an agricultural technology company, provides support to manufacturers on the Internet of Things (IoT) (Kılavuz & Erdem, 2019). Eshet Eilon company, on the other hand, uses X-rays to emerge the quality information of the fruit, when it will ripen or its nutritional value, by means of its spectral imaging machine (<https://www.btk.gov.tr/uploads/pages/arastirma-raporlari/akilli-tarim.pdf>).

**2.2.4. Germany:** CLASS company, carrying on its activities on agricultural machinery in Germany, monitors animal herds through sensors and informs the authorities via SMS if the animals are sick (Davutoğlu, 2021).

**2.2.5. United States of America:** The United States come into prominent for its investments in technology and learning to use technology. Accordingly, there are many institutes and sub-organizations in country's Federal Department of Agriculture (USDA). Under the leadership of the US Department of Commerce, the "Select USA" summit was held in 2016. At the summit, the importance of agricultural technologies was referred. With the support of the government, manufacturers use integrated systems that provide online humidity, temperature and harmful substance control in their

production areas. Even Though the USA meets 80% of the world almond production, almond is a product that requires a lot of water and production costs are increasing. In order to reduce the cost, soil analyzes made by placing moisture sensors on the trees and 20% savings were achieved. By means of a satellite sent to space by NASA, the amount of moisture in the soil is measured and the data obtained are presented to scientists. Again, the US-based tractor manufacturer John Deere reduced the fuel cost by 40% with the sensor added to the tractor (Kirmikil & Ertaş, 2020; Saygılı et al., 2019). Again, with an application called Scoutpro, developed in the USA, manufacturers can monitor their production sites in real time and access detailed information about the field (<https://www.btk.gov.tr/uploads/pages/arastirma-raporlari/akilli-tarim.pdf>).

**2.2.6. Canada:** Precision Hawk, operating in Canada, has developed a UAV sensor platform. Thanks to this sensor, manufacturers access necessary information on issues such as air pressure and wind speed (Davutoğlu, 2021).

**2.2.7. Japan:** Technology centers, universities and the private sector are at the forefront of smart agricultural practices in Japan. Japan's arable lands make up only 11% of its land, and the number of people working in the agricultural sector is gradually decreasing. However, Japan has increased agricultural production thanks to its investments in digital agriculture applications (<https://www.btk.gov.tr/uploads/pages/arastirma-raporlari/akilli-tarim.pdf>).

**2.2.8. Taiwan:** YesHealth IFarm in Taiwan has a fully controllable system. This production plant has 14 floors and vertically structured. While controlling the temperature, air and humidity, The nutrient absorption of plants is investigated by playing classical music in the facility. (Davutoğlu, 2021).

**2.2.9. Tanzania:** The “Connected Farmers” project was implemented in Tanzania with the contributions of Vodafone company. With this project, more than 30,000 manufacturers become organized organize through their smartphones in an area of hundreds of kilometers. In addition, thanks to this mobile phone application, agricultural industry companies can register producers, open loans, make payments to them or deliver the products collected from hundreds of producers to the processing area more effectively (<http://cv.ankara.edu.tr/duzenleme/kisisel/dosyalar/23112016172248.pdf>).

### **3. DIGITAL APPLICATIONS IN TURKISH AGRICULTURE**

Turkey is one of the ten largest agricultural producers in the world and the largest in Europe. In addition, considering its closeness to the European, Middle East and Central Asian markets, its relatively young population and the presence of agricultural land, it is clear that Turkey has an substantial agricultural potential. However, in order to use this potential at the highest level, it is necessary to give importance to digitalization in agriculture and to expand digitalization. In this direction, it would be beneficial to integrate information and communication technologies into all processes of agricultural production (Pakdemirli et al., 2021).

On the other hand, While Turkey has a serious potential in terms of agricultural activities, is a country can not use this potential sufficiently. In 2020, approximately 18% of the working population was employed in the agricultural sector and this accounted for 6.6% of the gross domestic product (GDP). In the same year, the contribution of the sector to GDP was 47.3 billion dollars. In 2020, Turkey became the world leader in the production of dried apricots, hazelnuts, quince and figs.

The annual average growth rate in the agricultural sector between 2003 and 2020 was determined as 2.5%. However, when the agricultural data refer to Turkey is analyzed, it is seen that the increase in agricultural GDP is lower than the general GDP increase (Oğul, 2022). On the other hand, considerable losses have been seen in our agricultural lands in recent years and our title of being a self-sufficient country in agriculture that we have for many years have been lost (Kaya, 2019). The Agriculture Law was took in effect on 18 April 2006 in order to determine and implement the necessary policies in order to increase the efficiency of the agricultural sector. There are some articles in the law that can be associated with agricultural transformation in particular. These;

- Ensuring product diversity in agriculture,
- Increasing productivity and quality in production,
- Development of product and agricultural input markets,
- Increasing competitiveness
- Making production site usage plans,
- Ensuring product-market integration, are classified (Sezer, 2022).

Therefore, it is clear that the use of digital agriculture applications in the execution of many agricultural activities, from planting to harvesting, from agricultural spraying to irrigation from fertilization to weeding, soil, plant and animal health, or marketing of products, is important in realizing the above-mentioned items related to agricultural transformation. These,

1. *In agricultural machinery;* Combines harvester with sensors used for harvesting are regarded as autonomous agricultural machines that inform the producers about the estimated harvest time, the water status and mineral level of the soil, or when fertilizer should be applied.
2. *In greenhouse activities;* Smart greenhouse systems are used for remote access in irrigation and air conditioning, balancing the greenhouse temperature, determining the pH value and radiation level.
3. *In product cultivation;* smart irrigation systems, drones used in spraying, seeding and irrigation, precision seeding equipment and robots used in pest control or weed removal are regarded as counted.
4. *In animal breeding;* The animal tracking system used to monitor the health and general condition of animals and to analyze the data obtained via computer, and smart traps used for the detection of pests are regarded as counted (Baran & Ersoy Karaçuha, 2021).

Ercan et al. (2019) evaluated agriculture 4.0 practices in Turkey through SWOT analysis. Their findings are given in Table 2.

**Table 2:** SWOT Analysis on Agriculture 4.0 Practices in Turkey

<b>Strength</b>	<b>Weaknesses</b>	<b>Opportunities</b>	<b>Threads</b>
Presence of organizations offering smart agriculture technologies	High average age of producers	Predominance of young population	Smart agriculture practices, causing a radical change, in the industry
Producers are inclined to cooperate with operating smart agriculture organizations	Inadequacy to use information technologies	Of smartphones, using widespread	Problems adapting to change that will be realized
Producers have positive opinions about the smart agriculture practices they use.	Lack of awareness for smart farming practices	Agricultural and technology organizations' requests for the necessary investment in education and R&D studies	Employment issues of agricultural workforce
The existence of certificate programs on smart agriculture technologies and the increasing interest in these programs	Foreign dependency in technology and infrastructure problems especially in rural areas	Demands for the forming of state policies for the spread of smart agricultural practices	Poor management of The state's to the process properly
Carrying out smart agriculture studies by public institutions	The fact that the lands have a fragmented structure and the agricultural enterprises are small-scale	Smart agriculture practices are to create employment opportunities in many sectors such as insurance.	Challenges encountered in intersectoral integration

On the other hand, the technological transformation desired to be realized in the agricultural sector in Turkey can be achieved not only with the producers, but also with the cooperation of all stakeholders consisting of the public and private sectors, unions and cooperatives and universities (Kaya,

2019). Below, some digital applications carried out in the agricultural sector in Turkey have been tried to be discussed on the basis of sectors.

### **3.1. Public Sector**

#### **3.1.1. Ministry of Agriculture and Forestry**

The Ministry of Agriculture and Forestry is the primary government actor responsible for agricultural production and the most prominent investor in Turkish digital agricultural solutions. Within the Ministry, the General Directorate of Agricultural Research and Policies is responsible for conducting research; the General Directorate began digital agriculture research programs in 2002. Some of these programs include: Determination of Variables Affecting the Yield in Cereal Cultivation Areas Using Precision Agricultural Techniques; Field Specific Variable Rate Fertilizer Application in Maize Production in the Çukurova Region Using Information Technologies Supported by Satellite Images; and the Integrated Project for Planning, Development, and Dissemination of Precision Agricultural Applications in Crop Production.

The Ministry also supports research programs conducted by ASELSAN, the largest defense electronics company in Turkey, with the aim of transferring the use of unmanned aerial devices, communication platforms, and traffic and automation systems to the field of agriculture. Some of ASELSAN's projects to date include: Automatic Tractor Steering and Control System Development; Farm Management Systems Development, Image Processing Based Precision Agricultural Applications with Unmanned Aerial Vehicles, System Development for Monitoring and Tracking Wheat Losses During Harvest, and Development of the Intelligent Measurement Platform Prototype for Sheep and Goat Breeding.

Also within the Ministry of Agriculture and Forestry, the Integrated Control System Department under the General Directorate of Agrarian Reform is responsible for several national datasets and the digital services they support, including:

- National Geographic Information Systems
- Farm Accountancy Data Network
- Land Parcel Identification System
- Integrated Management and Control System
- Agricultural Information Network
- Farmer Registration System
- Animal Registration System
- Farmland Registration System
- Village Database (FAO, 2021).

### **DİTAP (Digital Agriculture Market)**

The Digital Agricultural Market (DİTAP) is a project developed by the Ministry of Agriculture and Forestry in order to ensure that the products produced by the farmer attain all buyers at value prices and that both the producer and the consumer win. It was put into effect service for Real / Legal persons on 29 April 2020. The system is preferred by real persons, private institutions and public institutions in the positions of producers and buyers. The distinguished brands of our country and Central Unions and Cooperatives are also actively using the Digital Agricultural Market (DİTAP) system.

All buyers and manufacturers can meet through the online platform. It is on course to become a digital market where all stages from the seed stage of a product to the consumer's table are followed, supply and production provided, and production planned. Thanks to the Agricultural Market (DİTAP), Herbal Products, Animal Products and Aquaculture Products can be

sold directly. In order to expand export channels, pre-season prices are predicted by making contracted production. New modules continue to be added to the system. Thanks to the Agricultural Land Rental Module, which is one of these modules, it is possible for producer farmers to rent their own lands using the Digital Agricultural Market (DİTAP) system. One of the aims of the Digital Agriculture Market is to enable farmers being producers to find wider markets for their products. In addition, it is the creation of a platform where consumers and tradesmen can buy any quality product they want directly from the manufacturer without intermediaries. In this way, a much more efficient trade environment is created for both farmers, consumers and tradesmen (<https://www.dijitaltarim.com/dijital-tarim-pazari-ditap-nedir/>).

### **3.2. Financial Sector- Denizbank**

In addition to agricultural consultancy, DenizBank provides information on weather information, grant-support news and product prices at harvest times, in order to enable farmers to grow their crops more efficiently, with the "Deniz'den Toprağa-From Deniz to Land" digital agriculture application. Anyone interested in agriculture is able to make use of the application free of charge, whether they are a customer of the bank or not. It has reached over 270 thousand downloads. Thanks to the algorithms in the application, the entire growth process of a product from sowing to harvest is animated in a virtual environment, and fertilization, spraying and irrigation suggestions specific to each stage are instantly communicated to the producers. Users receive the responses they need via the application or by calling from their registered phones, by sending their questions 24/7 to the team of agricultural engineers, who are experts in their fields, with another important function, "Ask the Engineer". Market and stock market prices, news, official announcements and rental tractor functions allow the

manufacturer to enable instant access to information and to share the equipment and machinery necessary for the continuation of production. With the Satellite Field Tracking menu developed in 2019, users are able to pursue the satellite images of their recorded fields via the application, and monitor the development level of their fields online. In this way, they can get suggestions from agricultural engineers regarding underdeveloped regions in the field and increase their productivity (Denizbank, 2022).

### **3.3. Private Sector**

#### **3.3.1. Toros Tarım**

Toros Tarım is a Tekfen Chemical Industry Group company. It carries on its activities in the fields of special fertilizers and organic/organomineral fertilizers apart from mineral fertilizers. In classical fertilizers, it holds 38% of the total installed production capacity in Turkey. The “Toros Farmer Smart Agriculture and Fertilization Application”, distinctively developed by Toros Tarım for smart mobile phones and tablets, providing to farmers free of charge. The application is an agricultural decision support application that gather weather forecast, soil and plant data to develop suggestion for farmers' activities (<https://www.toros.com.tr/tr/toros-kurumsal/duyurular/toros-ciftci-uygulamasi-nedir>). The application, activated operation at the end of 2016, receives the coordinates of the field where the farmer sows, the results of the soil analysis laboratory and the information of the plant to be sowed; produces a list of fertilizers that can be used based on plant and soil data. The application, estimating the stage of the plant by considering the planting date and weather information, informs when being time to apply the top fertilizer. In addition, it also offers the farmer a calendar of the dates when the weather conditions are most convenient for fertilization. The farmer using the application can follow the daily weather forecasts for his field, as well as access the frost, hail and storm warnings through the application. Thus, the

farmer, having sufficient time to take the necessary measures, is able to minimize the possible damage those will encounter. In addition, thanks to the right fertilization at the right time, productivity increase and cost efficiency are also provided. There are approximately 7000 farmers, 7500 fields and 800 dealers registered in the “Torosfarmer-Torosçiftçi” application ([www.skdturkiye.org](http://www.skdturkiye.org))

### 3.3.2. Doktor

Doktor is an agricultural technology company that aims to optimize the food ecosystem with intensive use of information and data-based decisions. For this purpose, Doktor develops digital agriculture solutions based on new technologies such as remote sensing, machine learning and Internet of Things for the entire agricultural value chain. It establishes effective distribution systems and business partnerships for the collective use of these technologies. ***Doktor’s Digital Agricultural Products*** are Agricultural Information Services, Agricultural Sensor Station, Digital Soil Analysis, Variable Rate Fertilizer Application, Orbit Scouting & Crop Health, Farm Management System and Digital Pest Trap (<https://www.doktar.com/en>).

*Agricultural Information Services;* ensures farmers to have easy access to technical information, agricultural updates, market prices, grant support information, and obtain more yields with less input per unit area.

*Agricultural Sensor Station;* Take effective spraying and irrigation decisions with hourly data from the field.

*Digital Soil Analysis;* It enables the farmer to make fast and profitable decisions by determining the chemistry of soil and fertilizer needs within five minutes.

*Variable Rate Fertilizer Application;* Variable rate application service aims to reach your yield potential by taking differences within the field into account.

*Orbital Scouting and Crop Health;* By using daily satellite images, the farmer monitors the health of his field and easily determines the problem areas.

*Farm Management System;* It records all financial and operational activities related to the farmer's agricultural business and monitors its profitability in the field.

*Digital Pest Trap;* Provides real-time tracking of pests (<https://www.doktar.com/>).

*Doktar's Corporate Services;* ensures that the enterprises operating in the agriculture sector to connect with the farmers and provide satisfaction and sales-enhancing solutions. It creates competitive advantage by agricultural technologies that are supported by business decisions with farmers' insight. Corporate services are Market Intelligence, Agricultural & Technical Support Line, Doktar CRM, Agricultural Social Media Management, Crop Detection & Crop Type Identification, Digital Field Days and Traceability in Agricultural Procurement.

### **3.4. Cooperatives- Agricultural Credit Cooperatives**

#### **3.4.1. Agricultural Credit Technology**

Agricultural Credit Technology is a subsidiary of Turkey Agricultural Credit Cooperatives. It provides software services, hardware supply and communication infrastructure services by developing original projects for institutions and organizations in the public and private sectors beside cooperatives (<https://www.tarimkredi.org.tr/kurumsal/%c4%b1stiraklerimiz/tarim-kredi-teknoloji/>). The company cooperates with Gazi University Technopark. The company's brands in the agricultural sector; <https://www.tkteknoloji.com.tr/kurumsal/hakkimizda/>

**ZİHA (Agricultural Unmanned Aerial Vehicle):** In the plant protection method with ZİHA, the discharged liquid holds on to the plant in much higher amounts, the fuel cost which is included in the input cost decreases, and the damage caused by the relevant vehicle or tractor to the product is eliminated. Since distribution can be made completely autonomously, many factors related to human error are also disabled (<https://www.tkteknoloji.com.tr/sectorler/tarim-teknolojileri/z%C4%B1ha-zirai-%C4%B1nsansiz-hava-araci/>).

**ÇDP (Farmer Support Platform):** It is a platform that enables the collection and interpretation of agricultural data such as meteorological data, soil analysis data, insect trap data, field health and phytosanitary data by establishing an agricultural data infrastructure that can be used by all farmers, and then proceed to the stage of processing the analysis (<https://www.tkteknoloji.com.tr/en/sectors/agricultural-technologies/cdp-farmer-support-platform/>).

**Multispectral Camera UAVs:** Image analysis and artificial intelligence methods are used on multispectral / hyperspectral, visible / infrared / thermal bands, satellite images, aerial photographs obtained from UAVs and imaging aircraft, and images taken from the ground. The solutions are used to optimize the use of pesticides, water and fertilizers in sensitive agricultural practices, as well as provide information to farmers about the plant development process, to make more accurate planning by making harvest estimates and to protect their products from damage with early warning (<https://www.tkteknoloji.com.tr/en/sectors/agricultural-technologies/multi-spectral-uav/>).

**ATS (Smart Tractor Systems):** Robotic and autonomous technologies are adapted to agricultural machinery and presented to the use of farmers in

order to carry out more precise and accurate agricultural operations. The aim is to minimize human-induced errors and reduce labor, as well as to obtain better quality products and higher efficiency. Smart Tractor Systems will be an auxiliary technology that will have automatic and manual options, helping farmers to navigate their routes between rows and over rows properly on the field (<https://www.tkteknoloji.com.tr/en/sectors/agricultural-technologies/ats-smart-tractor-systems/>).

### **3.4.2. KORBIS (Turkey's First Digital Cooperative)**

It is an application enabling the members of agricultural credit cooperatives to do their work with cooperatives in a mobile environment with the Cooperative Joint Information System (KORBIS). Thanks to KORBIS, fast and easy access was provided, and the infrastructure of transactions such as instant tracking of partner information, interactive information, online order-collection, subsidies, and cash and in-kind loan applications was created (<https://tarimkredi.org.tr/faal%c4%b1yetler/korbis/>). The life of the partners is eased with Reminder Services, Institution and Branch Information, Nearest Cooperative, Call Center, Opinion, Suggestion and Request Communication, Campaign Notification, HAYBIS (Animal Information System), Partner Information and Agricultural Weather and Early Warning System in the KORBIS application.

*Satellite Field Tracking System (TARKIP)*; In agriculture, remote sensing technologies have been developed in order to provide the inputs needed to adapt the crop and soil conditions suitable for the land as required by Precision Agriculture, to perform all production processes more effectively, to ensure a continuous flow of information from the land and to prevent possible risks. With the system, the development, yield, water and stress (nutrient deficiency, drug follow-up, chlorophyll deficiency, disease, etc.) status of the products in the production land and the problems occurring

in different parts of the land is able to be detected early and compared with previous seasons. With this program, which will create a decision support system in production, efficiency is possible to be increased and risks to be reduced. TARKİP; It facilitates the work of partners with Breeding Suggestions, Agricultural Notification and Warning, Field Based Analysis, Accurate Decision-Making Center Database, Artificial Intelligence Studies, Early Warning System and Easy User Interface (<http://www.korbis.org.tr/Korbis/Index#features>).

### **3.5. GSM Operators-Turkcell and Vodafone**

#### **3.5.1. Turkcell**

Turkcell's applications for farmers are "Turkcell Farmer's World", "Turkcell Agricultural Doctor", "Turkcell Village Weather", "Turkcell Farm Doctor" and "Turkcell Filiz". These services are paid and subscription required.

*"Turkcell Farmer's World"* contains regional announcements, the latest developments in agriculture, current news from the Ministry of Agriculture and the Chambers of Agriculture, 3-day agricultural weather conditions specific to the district, the highest and lowest temperatures, meteorological events (sunny, partly cloudy, torrential rain, etc.) ([https://m.turkcell.com.tr/servisler/ciftci-haber-paketi?place=related\\_services](https://m.turkcell.com.tr/servisler/ciftci-haber-paketi?place=related_services)).

*"Turkcell Agricultural Doctor"* A separate program is created for each field/product registration. It is offered in the period until the harvest; product-specific technical breeding program, field and product-specific fertilization program, district-based 5-day meteorological forecasts, regional early warnings against diseases and pests, discounted soil and product analysis; market information about agricultural products, market and stock market prices, and regional and product-specific incentives and support information

from the Ministry of Agriculture and Forest (<https://m.turkcell.com.tr/servisler/turkcell-tarim-doktoru-paketi>).

*"Turkcell Village Weather"* Information is given from the 3-day agricultural weather forecast of the village ([https://m.turkcell.com.tr/servisler/koy-hava-durumu?place=related\\_services](https://m.turkcell.com.tr/servisler/koy-hava-durumu?place=related_services)).

*"Turkcell Farm Doctor"* Subscribers who register their animals are provided with nutrition, basic information on disease prevention, personalized veterinary services on farm management and reproduction, and services for customer relations ([https://m.turkcell.com.tr/servisler/turkcell-ciftlik-doktoru?place=related\\_services](https://m.turkcell.com.tr/servisler/turkcell-ciftlik-doktoru?place=related_services)).

*"Turkcell Filiz"* It is an application that allows the user to get more detailed information about the field, is used with the ground-weather station and can receive instant data. With the application, information about the field can be obtained without going to the field, and written and verbal support can be obtained from agricultural engineers who are experts in their fields (<https://m.turkcell.com.tr/kurumsal/dijital-is-servisleri/iot-nesnelerin-internali/turkcell-filiz>). With Turkcell Filiz, we regularly monitor different values of soil and air through sensors and combine with algorithms created with artificial intelligence; It provides the producers with critical information such as what should be done for the development of the plant, the need for water and the risks of disease, via the mobile application, and enables them to take precautions. It protects its users from possible harm by giving early warning against diseases, heavy rain, frost. Contains breeding information for sixty different plants (<https://medya.turkcell.com.tr/bulletins/dijital-tarimda-yerli-ve-milli-urun-filiz/>).

### 3.5.2. Vodafone

*Digital Agriculture Solution (Vodafone);* provides you with 'best timing' suggestions for agricultural operations by analyzing the data for specific land established that receives from the air and soil through the sensors and modules it is in, and helps the farmer to avoid product loss and equipment damage by giving early warnings. It is a support and decision system that allows to reduce production costs such as electricity, pesticide and fertilizer amount while maximizing the yield from the field (<https://www.vodafone.com.tr/vodafone-business/cozumler/dijital-tarim-cozumu>).

*Vodafone Farmers Club;* It is a versatile program that includes special advantages and offers to farmers and their relatives. Vodafone Farmers' Club members are possible to access all news that they interest in (weather forecast, product-specific disease and risk information, market and stock market prices, agricultural grants and supports, news about the agricultural sector and free fair invitations) with the Farmer News Package (<https://www.vodafone.com.tr/cifteci-kulubu>).

*Vodafone Smart Village;* The smart village is a new generation rural life model that combines traditional farming methods with the possibilities of advanced technology, making it a sole goal to increase the efficiency of production with information and communication technologies and to raise the social life standards of the producer with qualified knowledge. In this model, it is aimed to increase the productivity and profitability of small producers (family farming) with technological agriculture. In this model, it is aimed to increase the productivity and profitability of small producers (family farming) with technological agriculture (<https://www.tabit.com.tr/vodafone-akilli-koy/>). The project has been continuing successfully for 5 years with the cooperation of Vodafone and TABİT. Among its purposes; Increasing productivity in agricultural production with information and communication

technologies, preventing migration and unemployment by making young people love farming, ensuring digitalization in villages, using new agricultural technologies in the world. With Vodafone Smart Village, the inequality of opportunity that is gradually increasing, the convenience brought by technology, access to information and thus the difference in efficiency will be balanced. It prevents migration and supports reverse migration, thanks to the fact that the studies carried out increase both the welfare and income of the farmer in the area.

Vodafone Smart Village had organized trainings for farmers and studied on access issue to technology so that products can be grown with high efficiency, health and residues free (<http://www.vodafoneakillikoy.com/uploads/files/VakRapor.pdf>).

## **CONCLUSION**

Increasing world population, climate change and environmental problems reveal the value of scarce resources and the importance of sustainability. For this reason, while countries allocate significant resources to research and development activities for these developments, especially affecting the agricultural sector, technological developments in agriculture are becoming increasingly common. On the other hand, the Pandemic process in the world has also contributed to the understanding and spread of the importance of digital developments. Today, digitalization has commenced to become a crucial element for businesses, in term of competition, preserving and developing their presence in the market. The pandemic process we experienced had also enabled this digital world to go into our lives more quickly. Today, digital applications are seen in every sector and every type of business. Especially in the agricultural sector; Digital applications are of more importance due to the unique characteristics of the sector, the behavior of the producers, the structure and operation of agricultural enterprises, and its

relation with agricultural input and industrial sectors based on agriculture. Digital applications are mostly developed for the needs of manufacturers. In the field research conducted by the Credit Registration Bureau for 2021, it was determined that 80% of the farmers use smart mobile phones (with internet) and they connect to the internet mostly via smart mobile phones (75%). These rates give an idea that Turkish farmers are able to access the information they wish via the internet at any time. With the globalization process and increasing competition, agricultural enterprises have commenced to take an much more interest in technological innovations in order to increase income and reduce costs in production.

With the using of advanced technologies in agriculture, enables farmers to produce more agricultural products at less cost and also to contribute to the production of safe, quality and sustainable food. In this scope, smart agriculture applications and many other information communication technologies (mobile phones, internet, satellite images, remote sensing, sound waves, robots, advanced machines, etc.) have commenced to be used at all stages from soil to fork in the agricultural value chain. In this way, the need for labor has begun to decrease and the way for data to be collected and processed more frequently and accurately has been paved (Ministry of Agriculture and Forestry, 2022).

With the developments in the world in the field of Agriculture 4.0, Turkey has accelerated its work in this field. In Turkey, having a high agricultural production potential, research and development activities in the field of agricultural technology have been developed with the support of both state policies, universities and research centers and the private sector in recent years. In addition, especially GSM companies; The number of companies producing agricultural equipment, R&D and software and their patent applications are increasing day by day. In this field, examples of cooperation

among private sector companies have started to be seen. Vodafone Smart Village, established in Aydın with the aim of supporting rural development in partnership with Vodafone Turkey and TABİT, is on its way to becoming the first smart village in the world and Turkey equipped with end-to-end digital Technologies (Saygılı et al., 2019).

In our country, the level of awareness of producers about what smart agriculture is and its benefits is gradually increasing. Private sector companies, the public, cooperatives and technology companies have a great role in this increase. Digital applications are becoming widespread among manufacturers. Some companies offer their digital applications as free of charge. However, there are some obstacles to the spread of digital applications.

There are problems with government support and access. Among them;

- Due to the limited technical knowledge of the farmer, problems in data reading and interpretation,
- the inadequacy of the educated population working in agriculture,
- the lack of information support to be provided by reliable institutions in the selection of technology to be used in agriculture,
- the lack of information support to be provided by the farmer to evaluate the technology and get the most suitable one, fragmented and small structure of agricultural lands.
- Due to the high costs of the use of agricultural technology, the use of agricultural technology is introduced, encouraged and implemented in the stages. (Saygılı et al., 2019).

In order to disseminate smart agricultural practices, incentives should be given to small-scale enterprises, farmers should be trained on digital agriculture, pilot studies should be visited to ensure that the facilities and

benefits provided by the practices are observed, young people should be encouraged to agriculture, cooperation between producers, universities, public and industry should be ensured, domestic companies producing smart agricultural products should be encouraged and supported.

In summary to increase the efficiency, productivity and competitiveness of the agricultural sector in our country, both in terms of producers and production, digital applications in agriculture should be prevalent.

## REFERENCES

- Baran, E. & Ersoy Karaçuha, M. (2021). Küresel İklim Değişikliğine Uyum: Akıllı Tarım Uygulamaları ve İş Sağlığı ve Güvenliği. II. Ulusal İş Sağlığı ve Güvenliği Öğrenci Kongresi, 3-4 Nisan, İstanbul.
- Chitradevi, B. & Srimathi, P. (2014). An Overview on Image Processing Techniques. *International Journal of Innovative Research in Computer and Communication Engineering*, 2 (11), pp 6466-6472.
- Choudhary, S. K., Jadoun, R. S. & Mandoriya, H. L. (2016). Role Of Cloud Computing Technology In Agriculture Fields. *Computer Engineering and Intelligent Systems*, 7 (3), pp 1-7.
- Çakır, A. & İşlek, F. (2021). Türkiye'nin Akıllı Tarım (Tarım 4.0) Potansiyeli. Kökten, K. and İnci, H. (Ed.), In book: Türkiye'de Organik Tarım ve Agro-Ekolojik Gelişmeler (pp.155-174) Chapter: 7 Publisher: IKSAD Publishing House.
- Çolak, A., Acar, A. İ. & Orel, O. Tarım endüstri 4.0 (Pdf belgesi). 10 Ağustos 2022 tarihinde <http://cv.ankara.edu.tr/duzenleme/kisisel/dosyalar/23112016172248.pdf> adresinden erişildi.
- Denizbank, (2022). Denizbank 2021 Annual Report, p. 53 (<https://www.denizbank.com/dokumanlar/95871.vdf>)
- Davutoğlu, N. A. (2021). Sanayi 4.0 Uygulamalarının Dünyadaki ve Türkiye'deki Sektörler Açısından Detaylı Analizi. *Journal of Social and Humanities Sciences Research*, 8 (67), pp 795-811.
- Ercan, Ş., Öztep, R., Güler, D. & Saner, G. (2019). Tarım 4.0 ve Türkiye'de Uygulanabilirliğinin Değerlendirilmesi. *Tarım Ekonomisi Dergisi*, 25 (2), pp 259-265.
- (FAO, 2021) Food and Agricultural Organization, Digital Agriculture Profile Turkey, <https://www.fao.org/documents/card/es/c/cb3954en/>
- Fitzgerald, M., Kruschwitz, N., Bonnet, D. & Welch, M. (2013). Embracing Digital Technology: A New Strategic Imperative. MITSloan Management Review Research Report, pp 1-12.
- Guban, M. & Kovacs, G. (2017). Industry 4.0 Conception. *Acta Technica Corviniensis-Bulletin of Engineering*, 1, pp 111-114.
- Kaya, M. (2019). Ağrı'nın Kalkınması İçin Akıllı Tarım (Tarım 4.0) Önerisi. *Akademik Bakış Dergisi*, 75, pp 130-156.
- Kaymak, A. M., Örnek, M. N. & Kahramanlı, H. (2019). Görüntü İşleme Teknolojilerinin Elma Bahçelerine Yönelik Kullanım Örneği. *Uluborlu Mesleki Bilimler Dergisi (UMBBD)*, 2 (1), pp 17-26.
- Kılavuz, E. & Erdem, İ. (2019). Dünyada Tarım 4.0 Uygulamaları ve Türk Tarımının Dönüşümü. *Social Sciences (NWSASOS)*, 14 (4), pp 133-157.
- Kırkaya, A. (2019). Akıllı Tarım Teknolojileri Uygulamaları. III. Uluslararası Farkındalık Konferansı, pp165-180.
- Kirmikil, M. & Ertaş, B. (2020). Tarım 4.0 ile Sürdürülebilir Bir Gelecek. *ICONTECH International Journal of Surveys, Engineering, Technology*, 14 (1), pp 1-12.

- Klein, M. (2020). İşletmelerin Dijital Dönüşüm Senaryoları-Kavramsal Bir Model Önerisi. *Elektronik Sosyal Bilimler Dergisi*, 19 (74), pp 997-1019.
- Ministry of Agriculture and Forestry, (2022). ([https://cdn.nys.tarimorman.gov.tr/api/File/GetFile/330/Sayfa/1416/1778/DosyaGaleri/17\\_tarimda\\_teknolojik\\_donusumler.pdf](https://cdn.nys.tarimorman.gov.tr/api/File/GetFile/330/Sayfa/1416/1778/DosyaGaleri/17_tarimda_teknolojik_donusumler.pdf)) Access: 15.08.2022
- Oğul, B. (2022). Tarımsal Destekler ve Tarımsal Üretim İlişkisi: Türkiye Ekonomisi Üzerine Ampirik Bulgular. *TEAD*, 8 (1), pp 44-56.
- Ozdogan, B., Gacar, A. & Aktas, H. (2017). Digital Agriculture Practices in The Context Of Agriculture 4.0. *Journal of Economics, Finance and Accounting (JEFA)*, 4 (2), pp 184-191.
- Özsoylu, A. F. (2017). Endüstri 4.0. *Çukurova Üniversitesi İİBF Dergisi*, 21 (1), pp 41-64.
- Pakdemirli, B., Birişik, N., Aslan, İ., Sönmez, B. & Gezici, M. (2021). Türk Tarımında Dijital Teknolojilerin Kullanımı ve Tarım-Gıda Zincirinde Tarım 4.0. *Toprak Su Dergisi*, 10 (1), pp 78-87.
- Pogorelskaia, I. & Varallyai, L. (2020). Agriculture 4.0 and The Role of Education. *Journal of Agricultural Informatics*, 11 (1), pp 45-51.
- Saygılı, F., Kaya, A. A., Tunalı Çalışkan, E. & Erdölek Kozal, Ö. (2019). Türk Tarımının Global Entegrasyonu ve Tarım 4.0. İzmir Ticaret Borsası, Yayın no:98, ISBN 978-605-137-710-0, Ocak 2019 / İzmir.
- Sezer, A. (2022). Türkiye’de Toplumsal Cinsiyet ve Tarımda Ücretsiz Aile İşçiliği. Yüksek Lisans Tezi. Pamukkale Üniversitesi Sosyal Bilimler Enstitüsü, Denizli.
- Sott, K. M., Furstenau, L. B., Kipper, L. M., Giraldo, F. D., Lopez-Robles, J. R., Cobo, M. J.Imran, M. A. (2020). Precision Techniques And Agriculture 4.0 Technologies to Promote Sustainability in The Coffee Sector: State Of The Art, Challenges And Future Trends. *IEEE Access*, 8, pp 149854-149867.
- Tutkunca, T. & Haydar, O. (2022). Kooperatiflerde Dijital Dönüşüm ve Kooperatif Çalışanlarının İş Süreçlerine Etkisi: Çukobirlik Üzerine Bir Çalışma. *Scientific Journal of Innovation and Social Sciences Research*, 2 (1), pp 57-68.
- Wolfert, S., Ge, L., Verdouw, C. & Bogaardt, M. (2017). Big Data in Smart Farming- A Review. *Agricultural Systems*, 153, pp 69-80.
- Zhai, Z., Martinez, J. F., Beltran, V. & Martinez, N. L. (2020). Decision Support Systems For Agriculture 4.0: Survey And Challenges. *Computers and Electronics in Agriculture*, 170, pp 1-16.
- <https://www.btk.gov.tr/uploads/pages/arastirma-raporlari/akilli-tarim.pdf> Access: 08.08.2022.
- <http://cv.ankara.edu.tr/duzenleme/kisisel/dosyalar/23112016172248.pdf> Access: 08.08.2022
- <https://www.dijitaltarim.com/dijital-tarim-pazari-ditap-nedir/> Access: 15.08.2022
- <https://www.toros.com.tr/tr/toros-kurumsal/duyurular/toros-ciftci-uygulamasi-nedir> Access: 10.07.2022
- [www.skdturkiye.org](http://www.skdturkiye.org), (2022). Business Council for Sustainable Development Türkiye [http://www.skdturkiye.org/files/yayin/surdurulebilir-tarim-ilkeleri-iyi-uygulamalar-rehberi\\_4.pdf](http://www.skdturkiye.org/files/yayin/surdurulebilir-tarim-ilkeleri-iyi-uygulamalar-rehberi_4.pdf) , Access:16.07.2022
- <https://www.doktar.com/en> Access: 18.08.2022

- <https://www.doktar.com/> Access: 18.08.2022
- <https://www.tarimkredi.org.tr/kurumsal/%c4%b1stiraklerimiz/tarim-kredi-teknoloji/>  
Access: 15.08.2022
- <https://www.tkteknoloji.com.tr/kurumsal/hakkimizda/> Access: 15.08.2022
- <https://www.tkteknoloji.com.tr/sektorler/tarim-teknolojileri/z%C4%B1ha-zirai-%C4%B1nsansiz-hava-araci/> Access: 15.08.2022
- <https://www.tkteknoloji.com.tr/en/sectors/agricultural-technologies/cdp-farmer-support-platform/> Access:15.08.2022
- <https://www.tkteknoloji.com.tr/en/sectors/agricultural-technologies/multispectral-uav/> Access: 15.08.2022
- <https://www.tkteknoloji.com.tr/en/sectors/agricultural-technologies/ats-smart-tractor-systems/>Access:05.08.2022
- <https://tarimkredi.org.tr/faal%c4%b1yetler/korbis/> Access: 05.08.2022
- <http://www.korbis.org.tr/Korbis/Index#features> Access: 05.08.2022
- [https://m.turkcell.com.tr/servisler/ciftci-haber-paketi?place=related\\_services](https://m.turkcell.com.tr/servisler/ciftci-haber-paketi?place=related_services) Access: 15.08.2022
- <https://m.turkcell.com.tr/servisler/turkcell-tarim-doktoru-paketi>  
Access: 15.08.2022
- [https://m.turkcell.com.tr/servisler/koy-hava-durumu?place=related\\_services](https://m.turkcell.com.tr/servisler/koy-hava-durumu?place=related_services) Access: 15.08.2022
- [https://m.turkcell.com.tr/servisler/turkcell-ciftlik-doktoru?place=related\\_services](https://m.turkcell.com.tr/servisler/turkcell-ciftlik-doktoru?place=related_services)  
Access: 15.08.2022
- <https://m.turkcell.com.tr/kurumsal/dijital-is-servisleri/iot-nesnelerin-interneti/turkcell-filiz> Access: 15.08.2022
- <https://medya.turkcell.com.tr/bulletins/dijital-tarimda-yerli-ve-milli-urun-filiz/>  
Access: 15.08.2022
- <https://www.vodafone.com.tr/vodafone-business/cozumler/dijital-tarim-cozumu>  
Access: 16.08.2022
- <https://www.vodafone.com.tr/ciftci-kulubu> Access: 16.08.2022
- <https://www.tabit.com.tr/vodafone-akilli-koy/> Access: 16.08.2022
- <http://www.vodafoneakillikoy.com/uploads/files/VakRapor.pdf>). Access: 16.08.2022
- [https://www.kkb.com.tr/Resources/ContentFile/KKB\\_2021\\_TARIMSAL\\_GORUNUM\\_SAHA\\_ARASTIRMASI.pdf](https://www.kkb.com.tr/Resources/ContentFile/KKB_2021_TARIMSAL_GORUNUM_SAHA_ARASTIRMASI.pdf) (Kredi Kayıt Bürosu), Access: 16.07.2022



## CHAPTER 9

### RECENT DEVELOPMENTS OF GRAFTING IN *CUCURBITACEAE*

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## INTRODUCTION

Grafting is a vegetative propagation method defined as a combination of the shoot part of the plant, known as the scion, and the root part, known as the rootstock (Goldschmidt, 2014). Although it is not known how grafting was first discovered, it is said that it started to be practiced in Asia and the Middle East in the 5<sup>th</sup> century BC, and after it was mentioned in the ancient Greek and Chinese texts and the Bible. Furthermore, the relationship between mistletoe, apple, and poplar, people have observed that different kinds of plants grow on each other, and the ideas of obtaining new plants by cutting and combining different plants since ancient times has been tried (Melnyk and Meyerowitz, 2015). In addition, between the year 384 and 322 B.C, Aristotle explained the techniques of grafting. In the years 1300–1500, new grafting techniques had been used in Europe. Even though, the physiology of grafting was not yet known in England in the 16<sup>th</sup> century, the effect of cambium on grafting had been well understood (Hartmann and Kester, 1991; Belmonte-Urena et al. 2020). Tachisi had introduced the splice grafting technique in 1917 and the oblique splice grafting had been started by Watanabe in 1923. In 1962, Bravenboer has studied inoculation in *Solanaceae* plants and formed the source of grafting in the sense of agriculture (Louvet, 1974). The demand for grafting has been increased with the prohibition of methyl bromide, which is a soil disinfectant (Miles et al. 2017). Grafting of vegetables in European countries has been first started in cucurbits and then in *Solanaceae* species in the 1980s and continued intensively (Belmonte-Urena et al. 2020). Inoculation in fruit-edible vegetables has been started in the early of 20<sup>th</sup> century (Yetisir et al. 2004). The first grafting studies have been started in Japan and Korea due to the demand for continuity in agricultural production but insufficient areas (Ertok and Padem, 2007). The first grafting against fusarium wilt in vegetables started with the grafting of watermelon (*Citrullus lanatus*) on

gourd (*Lagenaria siceraria*) rootstock in Japan in 1914 (Yetisir et al. 2004). Following the spread of grafting in watermelons, grafted plants began to be produced in eggplant in 1950, cucumber in 1960, tomato in 1970, and melon until the 1990s (Ulaş and Yetişir, 2016; Malik et al. 2021). In this section, general information about grafting in cucurbits, grafting physiology, incompatibility in grafting, rootstocks, and techniques used in grafting and up-to-date information on grafting are given.

### **1. GRAFTING IN CUCURBITS**

The *Cucurbitaceae* family includes about 1000 species of important vegetables that are grown economically all over the world such as melon, watermelon, cucumber, bitter melon, bottle melon, was melon, and pumpkin (Guo et al. 2020). The global production of cucurbit crops is 159.35 million tons grown in the different regions of world (Faostat, 2020). Grafting on vegetables has been used for more than two decades in China, Korea, Turkey, Israel, France, Italy, and some other European countries. In the year of 2019, 58 million plants were grafted in North America, 24% (13.5 million) of which were watermelons. Grafted vegetables are grown in ratio of 51.5% in Mexico, 35% in Canada, and 13.5% in the United States. Today, 99% of Korea, 94% of Japan, and 40% of China are using grafted plants in watermelon production (Thies, 2021). At the beginning of the 21<sup>st</sup> century, with the prohibition of methyl bromide, which is a soil disinfectant in the USA, suitable grafting materials have been studied. As a result of those research studies, it has been reported that the grafting materials to be used should be closely related and compatible. The plants should be observed after grafting until harvesting time and the rootstock should be tolerant or resistant to soil-borne diseases and pests, biotic and abiotic stress conditions, and should be chosen as materials that show homogeneous growth on a commercial scale (Belmonte-Urena et al. 2020). The use of the grafting method is increasing every year, especially in

watermelon, to reduce soil-borne diseases and pests, which are difficult and limited (Fallik and Ziv, 2020). Rootstock selection is important in the grafting method.

Grafting is widely applied, especially in *Cucurbitaceae* and *Solanaceae* plants (Lee et al. 2010; Bie et al. 2017). Gourd (*Lagenaria siceraria* L.) and watermelon (*Citrullus amarus* Schrad.) rootstocks are used in limited numbers, while interspecies hybrids [*Cucurbita maxima* (Duchesne) x *C. moschata* (Duchesne ex Poir)] are commonly used rootstocks (Paris, 2015). Cucurbits are usually grafted onto the same species (*C. melo* L.) or cross-species like *Cucurbita moschata* × *Cucurbita maxima* hybrid rootstocks (King et al. 2010). The effect of *Lagenaria siceraria*, bitter melon (*Momordica charantia*) and ridge gourd (*Luffa acutangula*) rootstocks on cucumber was investigated. It has been determined that gourd rootstocks gave higher yields and had few dead plants compared to other rootstocks (Noor et al. 2019). It has been reported that the interspecific hybrid rootstocks (*C. maxima* × *C. moschata*), melon and watermelon genotypes, and gourds are salinity tolerant and usable in breeding programs, but *Luffa cylindrica* is susceptible to salt stress (Modarelli et al. 2020). Fruit quality, flesh color, and fruit firmness have also been positively affected by grafting technique. Plants grafted to interspecific *Cucurbita* rootstocks had more color intensity and more flesh firmness, but delayed color formation and an 8.8% decrease in TSS (Salar et al. 2014; Modarelli et al. 2020). Although the effect of rootstock and lineage genotype is important in grafting, rootstock-scion, lineage-environment, rootstock-environment and the interaction of these three are also very important (Thies, 2021). Grafting technique in cucurbits and used rootstocks are described in the following sections.

### 1.1. Watermelon

Generally, grafting technology with distinct rootstocks in watermelon has reached a good stage. The most common used rootstocks in watermelons are the interspecific hybrid pumpkins (*C. moschata* × *C. maxima*), gourd (*Lagenaria siceraria*) and *C. lanatus* var. *citroides* (Kong et al. 2014; Liu et al. 2015). Furthermore, pumpkin rootstocks improve fruit quality, yield, and resistance to disease and pests in watermelon (Xing et al. 2015) whereas grafting watermelon onto interspecific squash rootstock reduces TSS but increases yield, flesh firmness, and lycopene content (Kyriacou et al. 2020). In addition, *Cucurbita*, *Lagenaria*, and citron species and varieties have been tried as rootstocks for watermelon, and the authors reported that the best rootstock was the Nun9075 variety from *Cucurbita* hybrid (Kombo and Sari, 2019).

According to Davie et al. (2005), garfting did not change fruit shape of watermelon. The fruit flesh firmness is directly related to the amino acid called *Citrulline*, and it increases in fruit by grafting using zucchini cultivars (Soteriou et al. 2017; Fallik and Ziv, 2020). For watermelon, gourd (*Lagenaria siceraria* (Molina) Standl.) and interspecific hybrid pumpkin rootstocks (*Cucurbita moschata* (Molina) Standl. × *C. maxima* Duchesne) are very usable and durable rootstocks for resistance to *Fusarium oxysporum* f. sp. *niveum* (E. F. Smith) disease (Sheu et al. 2018; Thies, 2021). Additionally, *Cucurbita pepo* L., *Cucurbita maxima* Duchesne, *Cucumis melo* L. and *C. moschata* Duchesne rootstocks are applied to combat nematodes (*Meloidogyne incognita*) in watermelon varieties (López-Pérez et al. 2016). However, *C. colocynthis* and *Lagenaria* rootstocks are extremely sensitive to various diseases (Thies et al. 2010). The bottle gourd (*Lagenaria siceraria*), *Cucumis melo*, and squash (*Cucurbita pepo*) were used for *fusarium wilt* resistance and growth development in watermelon. For improvement of the

growth resistance to the low temperature, *C. maxima* x *C. moschata*, wax gourd (*Benincasa hispida*) and pumpkin (*Cucurbita moschata*), for nematode resistance *Sicyos angulatus* rootstocks are used (Mohanta et al. 2015).

### **1.2.Melon**

Recently, the interspecific hybrids of *Cucurbita moschata* and *Cucurbita maxima* and *Cucumis melo* species have been widely used as rootstock in melon (Karabulut et al. 2018). Several authors have stated that grafting techniques allow for the prevention of many stress factors and diseases in melon plants. For instance, when the wild watermelon *C. lanatus* var. *citroides* was used as rootstock against the necrotic spot virus the resistance was obtained between 0% and 20%, while the hybrid rootstock of *C. maxima* × *C. moschata* had approximately 100% resistance to the same disease (García-López et al. 2018). Furthermore, *Cucurbita* spp. are also used for fusarium wilts and growth promotion in open field grown melon, as well as *C. maxima* x *C. moschata* and *Cucumis melo* rootstocks for low temperature. On the other hand, for greenhouse grown melon, *Cucumis melo* and *Benincasa hispida* are utilized for fusarium wilts and growth development, and for low temperatures, the *C. maxima* x *C. moschata* rootstocks are used (Mohanta et al. 2015).

### **1.3.Cucumber**

Grafting methods in cucumber varieties have been shown to have greater prevalence due to the emergence of new disease strains and lower quality and yield from climate change. The main objectives of grafting cucumbers are to provide tolerant and resistant cucumber lines to the cold and fusarium wilt, and improve fruit appearance. Typically, cucumber has been grafted onto figleaf gourd (*C. ficifolia* Bouché), squash, interspecific hybrid squash, and pumpkin (Lee et al. 2010; Velkov and Pevicharova, 2016). Depending on the rootstock selection, most grafted plants have a higher total

fruit number and yield than other rootstocks or non-grafted plants (Goreta et al. 2014). Currently, grafted cucumbers with squash interspecific hybrid rootstocks provide earliness and increase yield by around 1.8% to 18.2 times compared to non-grafted cucumbers (Guan et al. 2020). In a study, *Cucurbita moschata* hybrid 360-3×112-2 and commercial *C. ficifolia* rootstocks have been used for salinity resistance, and it was stated that the most suitable rootstock was 360-3×112-2 hybrid rootstock (Zhou et al. 2010). In addition, TZ148, Nun 9075, and Local-3 rootstocks were found to improve the tolerance of cucumber plants to salinity stress conditions (Usanmaz and Abak, 2018). Moreover, the increase in the amount of dry matter, which is one of the most important criteria in the market and consumption phase, has been observed when the ‘figleaf gourd’ pumpkin rootstock was used (Zh et al. 2021). Generally, grafted plants produced higher yield, plant height (Farhadi and Rezaie 2015) and increased resistance to fusarium, which is one of the most important diseases, compared to ungrafted plants. However, there is not much increase in yield in soils devoid of diseases (Cardoso et al. 2010; Sabry et al. 2022). For fusarium wilts, growth development and low temperature in cucumber, fig. leaf (*Cucurbita ficifolia*, *C. maxima* x *C. moschata*, *Cucumis sativus*); for growth development and nematode resistance, *Sicyos angulatus* rootstocks are used (Mohanta et al. 2015).

## 2. GRAFTING PHYSIOLOGY

When rootstock and scion are cut, the plant perceives injury or fracture to its cell walls, which prompts defense and growth responses (Nuhse, 2012). After grafting, healthy cells adhere to opposing tissues and become stronger over time. Wrapping around the graft site with tape during grafting increases the physical pressure around the graft and supports the formation of the new grafted cell walls. For a while, the opening between the rootstock and root cells is V-shaped, and pectic substances accumulate in this opening. The

pectins, which accumulate and compress, increase the strength of the bond between the rootstock and the scion, and callus begins to form two days after grafting. Besides, the differentiation of the phloem and xylem occurs. The phloem, consisting of living cells, carries macromolecules, nutrients, water, and mineral substances to the callus, while the xylem, consisting of dead cells, the callus protrusions begin to form in the spaces between the rootstock and the scion. Meanwhile, the pectins inside begin to become thicker than the cell wall thickness. Four days after inoculation, callus cells fill the junction between rootstock and scion. Then, after five days, grafting formation occurs and pectins disappear (Jefree and Yeoman, 1983; Ertok and Padem, 2007; Melnyk et al. 2015). Plant hormones are effective for vegetative and generative growth in plants, and therefore, it is believed that they will also be effective in the connection between rootstock and scion (Aloni et al. 2010). Consequently, the use of various growth regulators to improve the grafting site and increase the grafting rate has begun to be investigated (Köse and Güleriyüz, 2006). Many studies have indicated that ABA has an effect on vascular tissue formation during the grafting (Nanda and Melnyk, 2018).

According to one view, the application of ABA suppresses the formation of genes that are activated by callus in the absence of callus (Pena-Cortes et al. 1989). In the case of ABA deficiency, there is a decrease in the suppression of genes that are active in callus. In this way, it is proven that ABA is directly effective in the formation of calli. In another view, ABA accumulated around the callus does not have any effect on the tissue but causes drying of the damaged tissues (Birkenmeier and Ryan, 1998). To combat salinity stress, resistant plants are obtained from grafting method application by using rootstocks with high ABA content levels (Etehadnia et al. 2008). Due to the auxin and cytokinin balance in the grafted plants being disturbed, more cytokines are transmitted to the shoot and the ratio of IAA is

decreased, which results in high growth development in the scion parts than in the rootstock (Aloni et al. 2010). Auxin also plays an important role in grafting during the grafted plant formation, as it allows vascular tissue formation and callus healing (Nanda and Melnyk, 2018). The low level of auxin differentiates the phloem in the callus, while its high level encourages the formation of both phloem and xylem (Aloni, 1980). The cotyledons are the source of oxygen, and the auxins that are produced from the cotyledons promote graft fusion in young plants (Bhalerao et al. 2002; Nanda and Melnyk, 2018). In other words, cytokinins are phytohormones with effects on cell division and plant growth (Nanda and Melnyk, 2018). Furthermore, cytokinins increase the formation of vascular tissues, but auxin is required for tissue reattachment. The coexistence of auxin and cytokinin allows reattachment of vascular tissues during grafting (Parkinson and Yeoman, 1982). Ethylene is effective in repairing a wound on the trunk (main stem), but has no significant effect on the grafted hypocotyl. However, it is synthesized around the graft junction (Yin et al. 2012; Nanda and Melnyk, 2018). Gibberellic acids contribute to the division and differentiation of cambium cells when they are supplied from the cotyledon. They support the closure of the callus and graft connection point repaired through the expanding cells (Asahina et al. 2002; Melnyk et al. 2015).

### **3. INCOMPATIBILITY IN GRAFTING**

The difference between incompatibility and compatibility is not known; nevertheless, it can be explained as the union and development of two plant parts to become a new plant (Belmonte-Urena et al. 2020). Incompatibility in grafting is manifested by being incapable of coexisting at the connection point between the rootstock and the scion, the un-growing grafted plant, and the premature death of the rootstock and the scion. This situation in grafting technique has been reported many times by several researchers, and this may

cause many unharvestable fruits (Malik et al. 2021). Yetisir and Sari, (2003) used 10 different rootstocks for watermelon and reported that *Lagenaria* type rootstocks were compatible with watermelon, and they stated that it appeared at a lower rate of incompatibility detected towards the end of vegetative development in *Cucurbita* rootstocks. Generally, it should be determined whether the plants to be used have the ability to be combined with each other before processing the grafting method. Although there are different opinions, the rate of graft fusion increases botanically in closely related plants (Kaşka and Yılmaz, 1974). Commonly, rootstocks with a strong root system and a positive affect on yield and quality should be selected (Suansia and Samal, 2021).

The grafting method, maintenance conditions, and nutritional status of the rootstock and scion are important in grafting (Philippines, 1990; Ertok and Padem, 2007). In the case of low compatibility, besides the decrease in quality and yield of the obtained product, deaths in plants occur (Lee, 1994). It was observed that the level of incompatibility decreased when hybrid rootstocks of the same genus and species were used, while the level of incompatibility increased in rootstocks between different genera. Therefore, the greater the taxonomic distance, the better the discrepancy (Pina et al. 2017). Compatibility in grafting depends not only on the appropriate combination of rootstock and scion but also on the anatomical, physiological, and genetic characteristics shared between rootstock and scion, as well as the environment and plant growing environment (Andrews and Marquez, 1993; Yarsi et al. 2010). With the incompatibility, the quality parameters, especially the internal values of the fruit decrease, while the fruit size is not affected (Lee and Oda, 2003). In a recent study, by examining various parameters, it was stated that physical parameters such as fruit length, diameter, and fruit rind thickness were not significantly affected, and also that compatibility in grafting is about

taxonomy (Nemeth et al. 2020; Malik et al. 2021). For instance, grafting onto *Luffa cylindrica* and melon, using melon scion is more compatible than pumpkin and wax guard (*Benincasa hispida*) (Wei et al. 2006); therefore, it has been identified that the closer the diameter of the hypocotyl to the diameter of the squash rootstocks, the less incompatibility occurs (Oda et al.1993). Although the compatibility in grafting depends on the rootstock combination, especially for cucurbits, this situation occurs in two different stages. The first stage is the formation of vascular connections within two weeks after grafting, and the second one is the occurrence of compatibility or incompatibility four weeks after grafting (Edelstein et al. 2004; Aloni et al. 2008). Accordingly, if there is an incompatibility in the grafting, the water uptake from the roots decreases, and the root-sugar concentration is reduced within 24 days after grafting (Aloni et al. 2008).

In a study conducted by Aloni et al. (2006) on melons, it was specified that the temperature factor was effective on inoculation in grafted plants. Reactive oxygen species (ROS) formation increases with grafting, while auxin formation decreases. This means that when the hormonal balance is disrupted, an incompatibility occurs directly. Reducing the incompatibility rate during grafting may be achieved by using the recognition of ROS activities appearing in various species. While cucumber and pumpkin are compatible, *Luffa cylindrica*, wax gourd (*Benincasa hispida*), bottle gourd (*Lagenaria siceraria*), bitter gourd (*Momordica charantia*), and watermelon are incompatible with melon (Xiong et al. 2021). Various studies on grafting incompatibility are limited to the use of pumpkin and gourd rootstocks. Anatomical studies are incomplete and need to be improved and developed for the evaluation of incompatibility among all *Cucurbitaceae* species (Xiong et al. 2021).

## **4. GRAFTING'S BENEFITS AND DRAWBACKS**

Grafting is a technique with potential and is widely preferred in *Cucurbitaceae* crops to eradicate the origins of soil-borne diseases (Louws et al. 2010). Rootstock and scion are effective on fruit quality. Some researchers found that grafting did not affect fruit quality (Matsumota, 1980; Moreno et al. 2016), while others reported that the quality was impaired by reducing flavor and fruit flesh staining and necrosis (Koutsika et al. 2002; Soteriou et al. 2016) and have also reported a positive effect in terms of taste and fruit texture (Buitelaar, 1987; Chung et al. 1997; Garcia et al. 2004; Colla et al. 2006; Melnyk, 2017; Ozbahce et al. 2021). In particular, during fruit development in the watermelon plant, the shape and size of the fruit were examined, and the study's results demonstrated that fruit growth and development are completed in three stages, such as the ovary development, the fruit development from the center and placental regions to the outside reaches 35 mm, and the cellular development of the fruit. Considering these stages, it is thought that grafting is likely to affect the internal or external quality of the fruit at any part of all these steps (Fallik and Ziv, 2020). Since rootstock and scion interact in the grafted plant, it has been determined that there are differences in the formation dates and levels of female flowers (Friedlander et al. 1977). More female flowers were formed in the watermelon grafted onto the *Lageneria siceraria* rootstock than in the ungrafted watermelon plant, but the flowers were formed later. The same result was reported in cucumber plants (Yamasaki et al. 1994; Satoh 1996).

### **4.1. Advantages**

Biotic stress conditions affect yield by 70% and abiotic stress factors by 30%. At this stage, various researchers have thought that constantly changing climatic conditions will increase these effect rates on crop production. According to the findings of these issues, grafting can provide

tolerant and resistant new cultivars to these stress factors (Kyriacou et al. 2020). It has been stated that plants that are cold-tolerant contain more linolenic acid and phospholipids than those that are not. For instance, cold stress affects various cucurbit plants. To arrange this case, it is better to use plants that contain linolenic acid and phospholipids during grafting because these biochemical compounds are cold-tolerant in plants and they prevent the decrease of chlorophyll and soluble protein content of plants at low temperatures. It is therefore recommended that *C. ficifolia* should be used in grafting for its potential as a cold-resistant rootstock, and because it can provide a 3-trans-hexadecenoic acid phosphatidyl glycerol content increase in the leaves, which normally occurs when soil temperatures increase (Horvath et al., 1983; Rivero et al., 2003; Malik et al. 2021). Therefore, photosynthetic activity, stomatal conductivity, and carbon-oxygen balance decrease, and plant transpiration is also reduced, which occurs when soil contains too much water. It is suggested that interspecific hybrid rootstocks can be used (Malik et al. 2021). When there is too much water in the soil, disease resistance can also be achieved with interspecific hybrid rootstocks. For instance, in a study, hybrid rootstocks of *Cucurbita maxima* x *Cucurbita moschata* were used, and it was reported that grafted melon plants were protected from soil-borne pathogens and higher yields were obtained in comparison to the control or ungrafted melon plants (Ozbahce et al. 2021).

**Table 1:** Advantages of grafting technique in cucurbits

Advantage	Reference
Increase in seed yield, seed germination and emergence rates.	Kombo and Sari, 2019
The yield increase between 60% and 90%	Colla et al. 2010; Chen et al. 2021; Dash et al. 2021
It provides protection against excess water in the soil.	Liao and Lin, 1996

Since the root systems of grafted plants are more developed, they can absorb water and nutrients better.	Tachibana, 1982; Schwarz et al. 2010; Roupael et al. 2008; Colla et al. 2011; Ozmen et al. 2015; Huang et al. 2016; Bertucci et al. 2018
The increase of the fruit rind thickness, the possibility of damage during transportation after harvest decreased.	Roupael et al. 2010; Moreno et al. 2016; Alan et al. 2018
The environmental pollution decreased as the use of suitable and durable rootstocks that provide plants tolerant or resistant to the diseases and pests increased, and the use of several chemicals that infected the soil decreased in amount.	Otani and Seike, 2007; Ulaş and Yetişir, 2016
Due to the strong root systems of grafted seedlings, plant growth and performance are positively affected, resulting in an increase in yield.	Lee, 1994; Yetisir and Sarı, 2003, Miguel, 2004; Davis et al. 2008; Yetisir and Uygur, 2010
Increase in resistance to soil-borne diseases.	Nisini et al. 2002; Davis et al. 2008; King et al. 2010; Lee et al. 2010; Louws et al, 2010; Villocino and Quevedo, 2015; Fallik et al. 2016; Kyriacou et al. 2016; Melnyk, 2017; Cardarelli et al. 2020
Tolerance can also be provided to biotic factors such as soil-borne pathogens and pests, and abiotic stress conditions such as drought, cold, temperature, salinity and heavy metal toxicity.	Roupael et al. 2008; Colla et al. 2010; Fallik and Ilic, 2014; Kyriacou et al. 2017; Melnyk, 2017; Gaion et al. 2018; Allevato et al. 2019; Singh et al. 2020
Early yield	Takahashi et al. 1982; Belmonte-Urena et al. 2020
The increase of carotenoid content.	Chen et al. 2021

#### 4.2. Disadvantages

Although grafting is used in disease control, there is a possibility of disease transmission (Goldschmidt, 2014). Transmission of viral diseases by root and grafting incompatibility are the major disadvantages in grafting programs (Epstein, 1978; Melnyk and Meyerowitz, 2015). The tools and equipment used in grafting also have an effect on the spread of viral and bacterial diseases (Bausher, 2013). Table 2 shows some disadvantages of grafting .

**Table 2.** Disadvantages of grafting method in cucurbits

<b>Disadvantage</b>	<b>Reference</b>
Decrease in nutritional values such as acidity and TSS (total soluble solids) .	Colla et al. 2010
The cost of grafted plants is higher than non-grafted plants, thus limiting the spread of the grafting program.	Singh et al. 2017; Devi et al. 2020; Fallik and Ziv, 2020
Depending on the rootstock and scion, but some rootstocks such as pumpkin, gourd and wax gourd cause a delay in plant flowering.	Yamasaki et al. 1994; Maurya et al. 2019
Transmission to plants occurs especially in viral diseases.	Epstein, 1978; Melnyk and Meyerowitz, 2015
Since the effect of rootstock and scion differs according to the combination and environmental conditions, difficulties may be experienced in the formation of the targeted product.	Goto et al. 2013; Singh et al. 2017
Since the diameters of the stems of the rootstock and scion do not match and the person who will perform the grafting cannot adjust this alignment, the success of the graft is adversely affected.	Lee et al. 2010

## 5. GRAFTING TECHNIQUES

There are certain methods developed according to the species in grafting. Grafting methods vary depending on the type of plant to be used, workers' knowledge, and conditions of grafting sites (Kyriacou et al. 2020). Hole insertion/terminal/top grafting, pin grafting, tube/one cotyledon/splice/slant grafting, tongue approach grafting, cleft/side insertion/approach grafting, and double grafting are the most grafting methods used in cucurbits (Lee et al. 2010; Malik et al. 2021).

**a) Hole insertion/terminal/top (HIG):** It is the method used mostly in *Cucurbitaceae*. In this method, the rate of retention in the grafted plants is high, and the application of the grafting is easy. After the growth point of the rootstock is taken, it is drilled at an angle of 45 degrees and the scion is cut to a length of 7-8 mm. Then the scion is placed in the hole of the rootstock. It is less costly in terms of labor because there is no need for clipping and root cutting (Hang et al. 2005; Kubota et al. 2008; Mohanta et al. 2015).

**b) Pin grafting:** In this method, which is similar to the one used in the cotyledon grafting method, a pin is used instead of a graft clip. The cotyledons are cut at an angle of 45 degrees on both the rootstock and scion. And the ceramic pins are used for joining (Mohanta et al. 2015).

**c) Tube/one cotyledon/splice/slant grafting:** This method is the most widely used method in cucurbits on a commercial scale and was developed for grafting robots (Dash et al. 2021; Sakata et al. 2007). When the rootstock and scion are at the same size, one of the two cotyledon leaves is cut at an angle of 45 degrees and the other cotyledon is cut from the base. Then, they are fixed by being joined on top of each other and stored at 25 degrees with 100% humidity for 3 days (Lee and Oda, 2003).

**d) Tongue approach grafting (TAG):** It is highly preferred due to its ease of use and low moisture requirement after grafting, and it is often used in cucurbits and eggplants. Rootstock seeds are sown after 10–13 days of scion seeds in cucumber, 7–10 days in zucchini and 1 week in other species. After the first true leaves are formed, the rootstock and the scion are cut at a 45-degree angle and attached to each other with a graft clip (Lee and Oda, 2003; Hassell et al. 2008; Mohanta et al. 2015; Dash et al. 2021).

**e) Cleft/side insertion:** This grafting method, which is more common in cucurbits and tomatoes, is important for its ease of application and has a pivotal role in high resistance to soil-borne diseases. After the growth point of

the rootstock is removed, it is cut and kept open with the help of toothpicks. A scion cut at an angle of 45 degrees is placed in the open area (Buzi et al. 2002; Amadio, 2004; Mohanta et al. 2015).

**f) Double grafting:** When there is no good rootstock for the shoot, a rootstock suitable for the desired characteristics is selected and grafted onto another good rootstock. It is not a very common method because of its low retention rate and high cost (Mohanta et al. 2015).

Generally, in grafting programs the rootstock grows again and competes with the scion, and the success of the graft is adversely affected, causing low yields (Devi et al. 2020). Although manual grafting is generally preferred to prevent rootstock regrowth, automated systems have also been developed (Memmott and Hassell 2010; Kubota et al. 2016). Even though a lot of research has been done in grafting and the use of robots in grafting in recent years, one of the factors that most affect the success of grafting is grafting techniques. With soil constraints, labor costs, and a lack of technical people who do grafting and develop technology, a tendency towards grafting robots has been achieved, especially in Asian countries (Yarsi, 2003; Kurata, 1994; Ulaş and Yetişir, 2016).

Moreover, grafting robots have been improved and developed recently. The first grafting robot was developed in China, and a plant was grafted in 4.5 seconds with a success rate of 95% (Kubota et al. 2008). Later, grafting robots were developed and marketed in Korea (Jung-Myung Lee et al. 2010) and Taiwan (Chen et al. 2010). Grafting robots are divided into two categories: manual, which require manual assistance; and fully automatic grafting robots, which do not require any intervention. Helper Robotech Korea and Conical System from semi-automatic grafting robots were used for *Solanaceae* plants, while Iseki was applied for *Cucurbitaceae*. In recent years, camera grafting robots have been introduced to take pictures of both rootstock and scion

junctions. These robots, which have a very low margin of error, are used in the Middle East, Asia, and Europe for precision inoculation. In this way, 1000 to 1050 plants can be grafted per hour (Kubota et al. 2016).

## **CONCLUSION**

Due to the challenges such as soil-borne diseases and pests, stress conditions, incompatibility, and lack of suitable rootstock materials, the yield and quality of cucurbits have significantly decreased. The chemicals used to prevent these stress conditions also cause environmental pollution. In addition, methods that may compete with diseases and pests against abiotic factors such as drought and salinity have been limited. Therefore, grafting is the most effective method to help and eradicate these challenges that hamper the plant's production. Owing to grafting, full resistance to diseases, especially fusarium, which is an important disease for cucurbits, is ensured, and adverse conditions can be minimized by choosing the appropriate rootstock against stress conditions. The mechanism of incompatibility and the physiology between rootstock and scion have not been fully resolved. In order to benefit from grafting at the maximum level, these issues should be investigated more in various studies. Furthermore, while breeding the new rootstocks, the focus should be on the problem of incompatibility as well as disease pest tolerance, and studies should be carried out to reveal its mechanism so that the cost of grafting can be reduced. In addition, it is recommended to carry out grafting studies on the shelf life of the fruit and the transfer of antioxidant substances for fruit quality with breeding studies to human health.

## REFERENCES

- Alan, O., Sen, F. and Duzyaman, E. (2018). The Effectiveness of Growth Cycle on Improving Fruit Quality for Grafted Watermelon Combinations, *Food Sci Technol*, 36, pp 270-277.
- Allevato, E., Mauro, R. P., Stazi, S. R., Marabottini, R., Leonardi, C. Ierna, A., Giuffrida, F. (2019). Arsenic Accumulation in Grafted Melon Plants: Role of Rootstock in Modulating Root-to-Shoot Translocation and Physiological Response, *Agronomy*, 9, pp 828.
- Aloni, R. (1980). Role of Auxin and Sucrose in the Differentiation of Sieve and Tracheary Elements in Plant Tissue Cultures, *Planta*, 150, pp 255- 263.
- Aloni, B., Karni, L., Deventurero, G., Levin, Z., Cohen, R., Katzir, N., Lotan-Pompan M., Edelstein, M., Aktas, H., Turhan, H., Joel, M. L., Horev, C. and Kapulnik, Y. (2006). Possible Mechanisms for Graft Incompatibility Between Melon Scions and Pumpkin Rootstocks. In IV International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops, Translating Seed and Seedling, 782, pp. 313-324.
- Aloni, B., Karni, L., Deventurero, G., Levin, Z., Cohen, R., Katzir, N., Lotan-Pompan, M., Edelstein, M., Aktas, H., Turhan, E., Joel, D. M., Orbe, C. and Kapulnik, Y. (2008). Physiological and Biochemical Changes at the Rootstock–Scion Interface in Graft Combinations Between *Cucurbita* Rootstocks and Melon Scion, *J. Hort. Sci. Biotechnol.* 83, pp 777-783.
- Aloni, B., Cohen, R., Karni, L., Aktas, H. and Edelstein, M. (2010). Hormonal Signaling in Rootstock–Scion Interactions, *Scientia Horticulturae*, 127, 2, pp 119-126.
- Amadio, A. (2004). Alternatives to Methyl Bromide Adopted for Cucurbit Production in Projects Funded by Montreal Protocol, Proc Fifth International Conference on Alternatives to Methyl Bromided, Lisbon, pp 71-74.
- Andrews, P. K. and Marquez, C. S. (1993). Grafting Incompatibility-Horticultural Reviews, *American Society of Horticultural Science*, 15, pp 183-232.
- Asahina, M., Iwai, H., Kikuchi, A. (2002). Gibberellin Produced in the Cotyledon is Required for cell Division During Tissue Reunion in the Cortex of Cut Cucumber and Tomato Hypocotyls, *Plant Physiol* 129, pp 201-210.
- Bausher, M. G. (2013). Serial Transmission of Plant Viruses by Cutting Implements During Grafting, *HortScience* 48, pp 37-39.
- Belmonte-Urena, L. J., Garrido-Cardenas, J. A., and Camacho-Ferre, F. (2020). Analysis of World Research on Grafting in Horticultural Plants, *HortScience*, 55, 1, pp 112-120.
- Bertucci, M. B., Suchoff D. H., Jennings, K. M., Monks DW, Gunter CC, Schultheis J. R., Louws, F. J. (2018). Comparison of Root System Morphology of Cucurbit Rootstock for Use in Watermelon Grafting, *HortTechnology* 28, pp 629-636.
- Bhalerao, R. P., Eklöf J, Ljung K. (2002). Shoot-Derived Auxin is Essential for Early Lateral Root Emergence in Arabidopsis Seedlings, *Plant J*, 29, pp 325-332.
- Bie, Z., Nawaz, M. A., Huang, Y., Lee, J. M. and Colla, G. (2017). Introduction of Vegetable Grafting, In *Vegetable Grafting, Principles and Practices*, ed. by

- Colla, G., Alfocea, F. P. and Schwarz, D. CABI Publishing, Wallingford, pp 1-21.
- Birkenmeier, G. F., and Ryan, C. A. (1998). Wound Signaling in Tomato Plants. *Plant Physiol* 117, pp 687-693.
- Buitelaar, K. (1987). Cultivars for the Very Early Culture of Melon. *Groenten en Fruit*, 42, pp 26-29.
- Buzi, A., Chilosi, G., Reda, R. and Magro, P. (2002). Le Principali Fitopatie che Colpiscono il Melone. *Colt. Prott.* 9, pp 31-45.
- Cardarelli, M., Roupael, Y., Kyriacou, M. C., Colla, G., Pane, C. (2020). Augmenting the Sustainability of Vegetable Cropping Systems by Configuring Rootstock-Dependent Rhizomicrobiomes that Support Plant Protection, *Agronomy*, 10, 1185.
- Cardoso, A. I. I., Salata, A. C., Magro, F. O., Bertolini, E. V. and Correa, C. V. (2010, August). Fruit Yield in Cucumber Grafted on Different Rootstocks, In XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010), International Symposium on 936, pp. 289-293.
- Chen, S., Chiu, Y.C., Chang, Y.C. (2010). Development of a Tubing Grafting Robotic System for Fruitbearing Vegetable Seedlings, *Applied Engineering in Agriculture*, 26, 4, pp707-714.
- Chen, S., Li, Y., Zhao, Y., Li, G., Zhang, W., Wu, Y. and Huang, L. (2021). iTRAQ and RNA-Seq Analyses Revealed the Effects of Grafting on Fruit Development and Ripening of Oriental Melon (*Cucumis melo* L. var. *makuwa*), *Gene*, 766, 145142.
- Chung, H. D., Youn, S. J. and Choi, Y. J. (1997). Effects of Rootstocks on Seedling Growth and Prevention of Root Rot *Fusarium Wilt* (race J3) in Different Tomato Cultivars, *Journal of Korean Society of Horticultural Science*, 38, pp 327-32.
- Colla, G., Roupael, Y., Cardarelli, M., Massa, D., Salerno, A. and Rea, E. (2006). Yield, Fruit Quality and Mineral Composition of Grafted Melon Plants Grown Under Saline Conditions. *Journal of Horticultural Science and Biotechnology*, 81, pp 146-52.
- Colla, G., Roupael, Y., Leonardi, C., and Bie, Z. (2010). Role of Grafting in Vegetable Crops Grown Under Saline Conditions, *Scientia Horticulturae*, 127, 2, pp 147-155.
- Colla, G., Roupael, Y., Mirabelli, C., and Cardarelli, M. (2011). Nitrogen-Use Efficiency Traits of Mini-Watermelon in Response to Grafting and Nitrogen-Fertilization Doses, *Journal of Plant Nutrition and Soil Science*, 174, 6, pp 933-941.
- Dash, R., Jena, C., Pramanik, K., and Mohapatra, P. P. (2021). Vegetable Grafting: A Noble Way to Enhance Production and Quality.
- Davis, A. R., Perkins-Veazie, P., Sakata, Y., Lopez-Galarza, S., Maroto, J. V., Lee, S. G. and Lee, J. M. (2008). Cucurbit Grafting, *Critical Reviews in Plant Sciences*, 27,1, pp 50-74.
- Devi, P., Lukas, S., and Miles, C. (2020). Advances in Watermelon Grafting to Increase Efficiency and Automation. *Horticulturae*, 6, pp 88.

- Edelstein, M., Burger, Y., Horev, C., Porat, A., Meir, A. and Cohen, R. (2004). Assessing the Effect of Genetic and Anatomic Variation of Cucurbita Rootstocks on Vigour, Survival and Yield of Grafted Melons, *J. Hortic. Sci. Biotechnol.* 79,3, pp 370-374.
- Epstein, A. H. (1978). Root Graft Transmission of Tree Pathogens, *Ann. Rev. Phytopathol.* 16, pp 181-192.
- Ertok, R. and Padem, H. (2007). Grafting Physiology in Vegetables, *Derim*, 24(2), pp 20-26.
- Etehadnia, M., Waterer, D., De Jong, H. and Tanino, K. K. (2008). Scion and Rootstock Effects on ABA-Mediated Plant Growth Regulation and Salt Tolerance of Acclimated and Unacclimated Potato Genotypes, *J. Plant Growth Regul.* 27, pp 125-140.
- Fallik, E., and Ilic, Z. (2014). Grafted vegetables – the Influence of Rootstock and Scion on Postharvest Quality, *Folia Hortic* 26, pp 79-90.
- Fallik, E., Alkalai-Tuvia, S., Chalupowicz, D., Zutahy, Y., Zaaroor, M., Beniches, M. and Gamliel, A. (2016). Effects of Rootstock and Soil Disinfection on Quality of Grafted Watermelon Fruit (*Citrullus lanatus* L.): a Two-Year Study, *Israel Journal of Plant Sciences*, 63, 1, pp 38-44.
- Fallik, E., and Ziv, C. (2020). How Rootstock/Scion Combinations Affect Watermelon Fruit Quality After Harvest, *Journal of the Science of Food and Agriculture*, 100, 8, pp 3275-3282.
- Faostat. 2020. <https://www.fao.org/faostat/en/#data/QCL/visualize> (Date of access: 02.08.2022).
- Farhadi, A. and Rezaie, M. (2015). Evaluation of Quantitative and Qualitative Traits of Greenhouse Cucumber (*Cucumis sativus* L. 'Khassib') Grafted on Different Cucurbita Rootstocks, *Acta Horticulturae*, 1086, pp 279–283. doi:10.17660/ActaHortic.2015.1086.35
- Friedlander, M., Atsmon, D. and Galvn, E. (1977). The Effect of Grafting on Sex Expression in Cucumber, *Plant and Cell Physiology* 18, pp 1343-50.
- Gaion, L. A., Braz, L. T. and Carvalho, R. F. (2018). Grafting in Vegetable Crops: a Great Technique for Agriculture, *International Journal of Vegetable Science*, 24, 1, pp 85-10.
- Garcia, F. N., Carvajal, M. and Enrique, O. (2004). Grafting Union Formation in Tomato Plants, *Annals of Botany*, 93, pp 53-60.
- García-López, F., García-López, A., González-Eguiarte, D. R., Rodríguez-Macías, R., Zarazúa-Villaseñor, P. and Huitrón-Ramírez, M. V. (2018). Watermelon Production with Rootstocks in Soils Infested with the Necrotic Melon Spot Virus, *Rev Mexi Cien Agríc* 9, pp 577-587.
- Goldschmidt, E. E. (2014). Plant Grafting: New Mechanisms, Evolutionary Implications, *Frontiers in plant Science*, 5, pp 727.
- Goto, R., de Miguel, A., Ignacio Marsal, J., Gorbe, E. and Calatayud, A. (2013). Effect of Different Rootstocks on Growth, Chlorophyll a Fluorescence and Mineral Composition of two Grafted Scions of Tomato, *J Plant Nutr* 36, pp 825-835.
- Goreta Ban, S., Dumičić, G., Raspudić, E., Vuletin Selak, G. and Ban, D. (2014). Growth and Yield of Grafted Cucumbers in Soil Infested with Root-Knot Nematodes, *Chilean journal of agricultural research*, 74, 1, pp 29-34

- Guan, W., Haseman, D., and Nowaskie, D. (2020). Rootstock Evaluation for Grafted Cucumbers Grown in High Tunnels: Yield and Plant Growth, *HortScience*, 55,6, pp 914-919.
- Guo, J., Xu, W., Hu, Y., Huang, J., Zhao, Y., Zhang, L., Chien-Hsuni H. and Hoang, M. (2020). Phylotranscriptomics in *Cucurbitaceae* Reveal Multiple Whole-Genome Duplications and Key Morphological and Molecular Innovations, *Mol. Plant* 13, pp 1117–1133. doi: 10.1016/j.molp.2020.05.011.
- Hang, S. D., Zhao, Y. P., Wang, G. Y. and Song, G. Y. (2005). *Vegetable Grafting*, China Agriculture Press, Beijing, China.
- Hartmann, H.T. and D.E. Kester. (1991). *Propagacion de Plantas, Principiosy Practicas*. CECSA, Mexico City, Mexico.
- Hassell, R. L., Memmott, F., Liere, D. G. (2008). Grafting Methods for Watermelon Production, *HortScience*, 43, pp 1677-1679.
- Horvath, I., Vigh, L., van Hasselt, P. R., Woltjecs, J. and Kuiper, P. J. C. (1983). Lipidcomposition in Leaves of Cucumber Genotypes as Affected by Different Temperature Regimes and Grafting, *Physiologia. Plantarum*, 57, pp 532-536.
- Jefree, C. E., Yeoman, M. M. (1983). Development of Intercellular Connections Between Opposing Cells in a Graft Union, *New Phytologist*, 93, pp 491-509.
- Jung-Myung Lee, C., Kubota, S. J., Tsao, Z., Bie, P., Hoyos Echevarria, L. Morra, M. Oda. (2010). Current Status of Vegetable Grafting: Diffusion, Grafting Techniques, Automation, *Scientia Horticulturae*, 127, 2, pp 93-105.
- Karabulut, A., Aktaş, H. and Bekir, Ş. (2018). The Effects of Grafted Seedling Use on Yield and Quality in Greenhouse Melon Cultivation, *Süleyman Demirel Journal of the University of Science and Technology*, 22,3, pp 1223-1231.
- Kaşka, N., Yılmaz, M. (1974). *Horticultural Cultivation Technique*. pp 331-332.
- King, S. R., Davis, A. R., Zhang, X. and Crosby, K. (2010). Genetic, Breeding and Selection of Rootstocks for *Solaneaceae* and *Cucurbitaceae*, *Sci. Hortic.* 127, pp 106–111.
- Kong, Q. S., Chen J. L., Liu Y., Ma, Y. H, Liu, P., Wu, S.Y. and Bie, Z. L. (2014) Genetic Diversity of *Cucurbita* Rootstock Germplasm as Assessed Using Simple Sequence Repeat Markers. *Sci Hortic* 175, pp 150-155.
- Kombo, M. D. and Sari, N. (2019). Rootstock Effects on Seed Yield and Quality in Watermelon, *Horticulture, Environment, and Biotechnology*, 60,3, pp 303-312.
- Koutsika, S. M., Traka, M. E., Paroussi, G., Vayiatzis, D. and Paraoussis, E. (2002). The Cultivation of Grafted Melon in Greece, Current Status Prospects *Acta Horticulturae* 579, pp 325-30.
- Köse, C. and Güleriyüz, M. (2006). Effects of Auxins and Cytokinins on Graft Union of Grapevine (*Vitis vinifera*) New Zealand, *J. Crop Hortic. Sci.* 34, pp 145-150.
- Kubota, C., McClure, M. A., Kokalis-Burelle, N., Bausher, M.G. and Roskopf, E. N. (2008) *Vegetable grafting: history, use, and current technology status in North America*. *HortScience*, 43, 1664-1669.
- Kubota, C., Miles, C., and Zhao, X. (2016). Chapter 4. Automation in Vegetable Grafting Nurseries, in *Grafting Manual: How to Produce Grafted Vegetable*

- Plants, ed. By National Institute of Food and Agriculture, Washington, DC, pp. 1–5 .
- Kurata, K. (1994). Cultivation of Grafted Vegetables. II. Development of Grafting Robots in Japan, *HortScience* 29, pp 240-244.
- Kyriacou, M. C., Soteriou, G. A., Roupael, Y., Siomos, A. S. and Gerasopoulos, D. (2016). Configuration of Watermelon Fruit Quality in Response to Rootstock-Mediated Harvest Maturity and Postharvest Storage, *Journal of the Science of Food and Agriculture*, 96,7, pp 2400-2409.
- Kyriacou, M. C., Roupael, Y., Colla, G., Zrenner, R. and Schwarz, D. (2017). Vegetable Grafting: the Implications of a Growing Agronomic Imperative for Vegetable Fruit Quality and Nutritive Value, *Front Plant Sci* 8, pp 741.
- Kyriacou, M. C., Colla, G. and Roupael, Y. (2020). Grafting as a Sustainable Means for Securing Yield Stability and Quality in Vegetable Crops, *Agronomy*, 10, 12, pp 1945.
- Kyriacou, M. C., Soteriou, G.A. and Roupael, Y. (2020). Modulatory Effects of Interspecific and Gourd Rootstocks on Crop Performance, Physicochemical Quality, Bioactive Components and Postharvest Performance of Diploid and Triploid Watermelon Scions, *Agronomy*, 10, 1396.
- Lee, J. M. (1994). Cultivation of Grafted Vegetables I. Current Status Grafting Methods and Benefits, *Hort Science*, 29,4, pp 235-239.
- Lee, J. M. and Oda, M. (2003). Grafting of Herbaceous Vegetable and Ornamental Crops, *Hortic. Rev.* 28, pp 61-124.
- Lee, J., Kubota, C., Tsao, S. J., Bie, Z., Echevarria, P. H., Morra, L. and Oda, M. (2010). Current Status of Vegetable Grafting: Diffusion, Grafting Techniques, Automation. *Scientia Horticulturae*, 127, pp 93-105.
- Liao, C. T. and Lin, C. H. (1996). Photosynthetic Response of Grafted Bitter Melon Seedling to Flood Stress, *Env. And Exp. Bot* 36, pp 167-172.
- Liu, B., Ren, J., Zhang, Y., An, J., Chen, M., Chen, H., Xu, C. and Ren, H. (2015) A New Grafted Rootstock Against Root-Knot Nematode for Cucumber, Melon, and Watermelon, *Agron Sustain Dev* 35, pp 251-259.
- López-Pérez, M., Talavera, M. and Verdejo-Lucas, S. (2016). Differential Reproduction of *Meloidogyne incognita* and *M javanica* in Watermelon Cultivars and Cucurbit Rootstocks, *Plant Path* 65, pp 145-153 .
- Louvet, J. (1974). L'utilisation du Greffage en Culture Marichere, *PHM RevueHort*, pp 19-24.
- Louws, F. J., Rivard, C. L. and Kubota, C. (2010). Grafting Fruiting Vegetables to Manage Soilborne Pathogens, Foliar Pathogens, Arthropods and Weeds, *Sci. Hortic.* 127, pp 127-146. doi: 10.1016/j.scienta.2010.09.023
- Malik, A. A., Malik, G., Narayan, S., Hussain, K., Mufti, S., Kumar, A., Khan, F.A., Kumar, H. and Lone, S. (2021). Grafting Technique in Vegetable Crops-A Review, *Skuast Journal of Research*, 23,2, pp 104-115.
- Maurya D, Pandey AK, Kumar V, Dubey S, Prakash V. (2019). Grafting Techniques in Vegetable Crops: A Review, *International Journal of Chemical Studies*,7,2, pp 1664-1672.

- Matsumota, M. (1980). The Mechanism of Bloom Occurrence on the Surface of the Cucumber Fruits and Methods for its Prevention, Bulletin of Toyama Agricultural Experiment Station, 11, pp 29-35.
- Melnyk, C. W. and Meyerowitz, E. M. (2015). Plant Grafting, Current Biology, 25,5, pp 183-188.
- Melnyk, C. W., Schuster, C., Leyser, O., and Meyerowitz, E. M. (2015) A Developmental Framework for Graft Formation and Vascular Reconnection in *Arabidopsis thaliana*, Curr Biol 25, pp 1306-1318.
- Melnyk, C. W. (2017). Plant Grafting: Insights into Tissue Regeneration, Regeneration, 4,1, pp 3-14.
- Memmott, F. D. and Hassell, R. L. (2010). Watermelon (*Citrullus lanatus*) Grafting Method to Reduce Labor Cost by Eliminating Rootstock Side Shoots. Acta Hort., 871, pp 389-394.
- Miguel, A. (2004). Use of Grafted Cucurbits in the Mediterranean Region as an Alternative to Methyl Bromide. In: Proc. Fifth International Conference on Alternatives to Methyl Bromide, Lisbon, pp 151-156.
- Miles, C., Devi, P., Zhao, X., Guan, W. (2017). Grafting Manual: How to Produce Grafted Vegetables Plants, United States Department of Agriculture, National Institute Food and Agriculture. (<http://www.vegetablesgrafting.org>).
- Modarelli, G.C., Roupael, Y., De Pascale, S., Öztekin, G.B., Tüzel, Y., Orsini, F. and Gianquinto, G. (2020). Appraisal of Salt Tolerance Under Greenhouse Conditions of a *Cucurbitaceae* Genetic Repository of Potential Rootstocks and Scions, Agronomy, 10, pp 967.
- Mohanta, S., Prasad, B. V. G., Rahaman, S. and Bareilly, P. (2015). Vegetable Grafting, Journal of Agricultural Engineering and Food Technology. Print ISSN: 2350-0085; Online ISSN: 2350-0263, 2, Number 2; April-June, pp 104-108.
- Moreno B, Jacob C, Rosales M, Krarup K. and Contreras, S. (2016). Yield and Quality of Grafted Watermelon Grown in a Field Naturally Infested With Fusarium Wilt, HortTechnology 26, pp 453-459.
- Nanda, A. K. and Melnyk, C. W. (2018). The Role of Plant Hormones During Grafting. Journal of plant research, 131,1, pp 49-58.
- Nemeth, D., Balázs, G., Bodor, Z., Zaukuu, J. L. Z., Kovács, Z. and Kappel, N. (2020). Food Quality Attributes of Melon (*Cucumis melo* L.) Influenced by Grafting, Progress in Agricultural Engineering Sciences, 16, 1, pp 53-66.
- Nisini, P. T., Colla, G., Granati, E., Temperini, O., Crino, P. and Saccardo F. (2002). Rootstock Resistance to Fusarium wilt and Effect on Fruit Yield and Quality of Two Muskmelon Cultivars, Sci. Hort 93, pp 281-288.
- Noor, R. S., Wang, Z., Umair, M., Yaseen, M., Ameen, M., Rehman, S.U., Khan, M.U., Imran, M., Ahmed, W. and Sun, Y. (2019). Interactive Effects of Grafting Techniques and Scion-Rootstocks Combinations on Vegetative Growth, Yield and Quality of Cucumber (*Cucumis sativus* L.), Agronomy, 9, pp 288.
- Nuhse, T. S. (2012). Cell Wall Integrity Signaling and Innate Immunity in Plants, Frontiers in Plant Science, 3, pp 280.

- Oda, M., Tsuji, K. and Sasaki, H. (1993). Effect of Hypocotyl Morphology on Survival Rate and Growth of Cucumber Seedlings Grafted on *Cucurbita* spp, Japan Agricultural Research Quarterly, 26, pp 259-259.
- Otani, T. and N. Seike. (2007). Rootstock Control of Fruit Dieldrin Concentration in Grafted Cucumber (*Cucumis sativus*). J. Pestic. Sci. 32, pp 235– 242.
- Ozbahce, A., Kosker, Y., Gultekin, R., Gorgisen, C., Avag, K., Demir, Y. and Yucel, S. (2021). Impact of Different Rootstocks and Limited Water on Yield and Fruit Quality of Melon Grown in a Field Naturally Infested With Fusarium wilt, Scientia Horticulturae, 289, 110482.
- Ozmen, S., Kanber, R., Sari, N., and Unlu, M. (2015) The effects of Deficit Irrigation on Nitrogen Consumption, Yield, and Quality in Drip Irrigated Grafted and Ungrafted Watermelon, J Integr Agric 14, pp 966-976.
- Paris, H. S. (2015). Origin and Emergence of the Sweet Dessert Watermelon, *Citrullus lanatus*, Ann Bot 116, pp 133-148.
- Parkinson, M. and Yeoman, M. M. (1982). Graft Formation in Cultured, Explanted Internodes. New Phytol 91, pp 711-719. <https://doi.org/10.1111/j.1469-8137.1982.tb03350.x>.
- Pena-Cortes, H., Sánchez-Serrano, J. J., Mertens, R., Willmitzer, L. and Prat, S. (1989). Abscisic Acid is Involved in the Wound-Induced Expression of the Proteinase Inhibitor II Gene in Potato and Tomato, Proceedings of the National Academy of Sciences, 86, 24, pp 9851-9855.
- Philippines, R. (1990). A Preliminary Study of Graft Compatibility of Bittergourd Scion on Spongegourd and Bottlegourd Rootstock. ARC Training.
- Pina, A., Cookson, S. J., Calatayud, A., Trinchera, A. and Errea, P. (2017). Physiological and Molecular Mechanisms Underlying Graft Compatibility, in Vegetable Grafting: Principles and Practices, ed. by Colla, G., Pérez- Alfocea, F. and Schwarz, D., CABI, Wallingford, pp 132-154.
- Rivero, R. M., Ruiz, J. M. and Romero, L. (2003). Role of Grafting in Horticultural Plants Under Stress Conditions, Journal of Food, Agriculture and Environment, 1, pp 70-74.
- Rouphael, Y., Cardarelli, M., Colla, G. and Rea, E. (2008). Yield, Mineral Composition, Water Relations, and Water Use Efficiency of Grafted Mini-Watermelon Plants Under Deficit Irrigation, Hort Science;43, 3, pp 730-736.
- Rouphael, Y., Schwarz, D., Krumbein, A. and Colla, G. (2010). Impact of Grafting on Product Quality of Fruit Vegetables, Sci Hortic, 127, pp 172-179.
- Sabry, S., Ali, A. Z., Abdel-Kader, D. A. and Abou-Zaid, M. I. (2022). Histopathological and Biochemical Aspects of Grafted and Non-Grafted Cucumber Infected With Stem Rot Caused by *Fusarium* spp, Saudi Journal of Biological Sciences, 29,3, pp 1770-1780.
- Salar, N., Salehi, R. and Delshad, M. (2014). Effect of Grafting and Nitrogen Application on Yield and Fruit Quality of Grafted and Non-Grafted Melon, In I International Symposium on Vegetable Grafting 1086, pp. 225-230.
- Sakata, Y., Takayoshi, O. and Mitsuhiro, S. (2007). The History and Present State of the Grafting of *cucurbitaceous* in Japan, Acta Hort. 731, pp 159-170.
- Satoh, S. (1996). Inhibition of Flowering of Cucumber Grafted on Rooted Squash Stocks, Physiol. Plant. 97, pp 440-444.

- Schwarz, D., Roupshael, Y., Colla, G. and Venema, J. H. (2010). Grafting as a Tool to Improve Tolerance of Vegetables to Abiotic Stresses: Thermal stress, Water Stress and Organic Pollutants, *Scientia Horticulturae*;127, 2, pp 162-171.
- Sheu, Z. M., Cheng, H. C., Chiu, M. S., Yu, C. C., Huang, H. Y., Barchenger, D. W. and Kenyon, L. (2018, August). Evaluation of Cucurbit Rootstocks and Screening of Bitter Gourd Genotypes for Resistance to *Fusarium wilt*. In XXX International Horticultural Congress IHC2018: International Symposium on Tropical and Subtropical Vegetable Production: 1257, pp. 57-62.
- Singh, H., Kumar, P., Chaudhari, S., and Edelstein, M. (2017). Tomato Grafting: A Global Perspective, *HortScience* 52, pp 1328-1336.
- Singh, H., Kumar, P., Kumar, A., Kyriacou, M. C., Colla, G. and Roupshael, Y. (2020). Grafting Tomato as a Tool to Improve Salt Tolerance, *Agronomy*, 10, pp 263.
- Soteriou, G. A., Papayiannis, L. C., Kyriacou, M. C. (2016). Indexing Melon Physiological Decline to Fruit Quality and Vine Morphometric Parameters, *Scientia Horticulturae* 203, pp 207-215.
- Soteriou, G. A., Siomos, A. S., Gerasopoulos, D., Roupshael, Y., Georhiadou, S. and Kyriacou, M. C. (2017). Biochemical and Histological Contribution to Texture Changes in Watermelon Fruit Modulated by Grafting, *Food Chem* 237, pp 133–140.
- Suansia, A. and Samal, K. C. (2021). Vegetable Grafting: A Sustainable and Eco-Friendly Strategy for Soilborne Pest and Disease Management, *J. Pharmacogn. Phytochem*, 10, pp 1634-1642.
- Tachibana, S. (1982). Comparison of Root Temperature on the Growth and Mineral Nutrition of Cucumber Cultivars and fig Leaf Gourd [in Japanese with English summary], *Japan. Soc. Hort. Sci.* 1: pp 299-308.
- Takahashi, H., Satio, T. and Suge, H. (1982). Intergeneric Translocation of Floral Stimulus Across a Graft Monoecious *Cucurbitaceae* With Special Reference to the Sex Expression of Flowers, *Plant and Cell Physiology*, 23, pp 1-9.
- Thies, J. A., Ariss, J. J., Hassell, R. L., Olson, S. and Levi, A. (2010). Grafting for Management of southern Root-Knot Nematode, *Meloidogyne Incognita*, in Watermelon, *Plant Dis* 94, pp 1195-1199.
- Thies, J. A. (2021). Grafting for Managing Vegetable Crop Pests, *Pest Management Science*, 77, 11, pp 4825-483.
- Ulaş, F. and Yetişir, H. (2016). The History of Grafting in Vegetables and Its Development in the World and in Turkey, *Nevşehir Journal of Science and Technology*.
- Usanmaz, S. and Abak. K. (2018). Plant Growth and Yield of Cucumber Plants Grafted on Different Commercial and Local Rootstocks Grown Under Salinity Stress, *Saudi Journal of Biological Sciences*, 26, 6, pp 1134-1139.
- Wei, S. Y., Wu, Z. and Huang, J. (2006). Effects of Rootstocks on Growth and Photosynthetic Properties of Grafted Plants of Netted Melon, *Acta Agriculturae Shanghai*, 22, pp 114-17.
- Xing, W. W., Li, L., Ga, P., Li, H., Shao, Q. S., Shu, S., Sun, J. and Guo, S. (2015). Effects of Grafting With Pumpkin Rootstock on Carbohydrate Metabolism in Cucumber Seedlings Under Ca (NO<sub>3</sub>)<sup>2</sup> Stress, *Plant Physiol Biochem* 87, pp 124-132.

- Xiong, M., Liu, C., Guo, L., Wang, J., Wu, X., Li, L., Bie, Z., and Huang, Y. (2021). Compatibility Evaluation and Anatomical Observation of Melon Grafted Onto Eight Cucurbitaceae Species, *Frontiers in Plant Science*, pp 2276.
- Velkov, N. and Pevicharova, G. (2016). Effects of Cucumber Grafting on Yield and Fruit Characteristics, *Zemdirbyste-Agriculture*, 103, 4, pp 405-410.
- Villocino Jr, S., B. and Quevedo, M. A. (2015). Effects of Grafting on Flowering, Fruiting and Fruit Quality of 'Sweet 16' watermelon (*Citrullus lanatus* Thunb.), *Acta Horticulturae*, 1088, pp 469-472.
- Yarsi, G., 2003. Investigation of the Effects of Using Grafted Seedlings on Yield, Fruit Quality and Plant Nutrient Intake in Greenhouse Melon Cultivation, Ç.Ü. Graduate School of Natural and Applied Sciences, Ph.D. thesis, Adana. 149s.
- Yarsi, G., Sari, N. and Yetisir, H. (2010, August). Effect of Different Rootstocks on the Yield and Quality of Grafted Melon Plants, In XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 936, pp 411-416.
- Yamasaki, A., Yamashita, M. and Furuya, S. (1994). Mineral Concentrations and Cytokinin Activity in the Xylem Exudate of Grafted Watermelons as Affected by Rootstocks and Crop Load, *Journal of the Japanese Society for Horticultural Science*, 62, 4, pp 817-826.
- Yetisir, H. and Sari, N. (2003). Effect of Different Rootstock on Plant Growth, Yield and Quality of Watermelon, *Australian journal of experimental agriculture*, 43, 10, pp 1269-1274.
- Yetisir, H., Yarşi, G. and Nebahat, S. (2004). Grafting in Vegetable, *Bahçe*, 33, 1.
- Yetisir, H. and Uygur, V. (2010). Responses of Grafted Watermelon onto Different Gourd Species to Salinity Stress, *Journal of Plant Nutrition*, 33,3, pp 315-327.
- Yin H, Yan B, Sun J, (2012). Graft-Union Development: A Delicate Process That Involves Cell–Cell Communication Between Scion and Stock for Local Auxin Accumulation. *J Exp Bot* 63, pp 4219-4232.
- Zh, S. A., Dzhantassov, S. K. and Nussupova, A. O. (2021). Efficiency Applications of Domestic Pumpkin Rootstocks for Grafting Cucumber on Protected Ground, *Научный журнал «Доклады НАН РК»*, 2, pp 119-126.
- Zhou, J., Hu, H., Li, X., Zhao, R., Li, G. and Yang, P. (2010). Effects of Rootstock on Fruit Yield and Quality of Hydroponically Cultivated Grafted Cucumber Under Nacl Stress, *Acta Hort.*, 871, pp 63-70.

## CHAPTER 10

### THE AGRICULTURAL WASTE VALORIZATION AND SUSTAINABLE MUSHROOM CULTIVATION IN TÜRKİYE

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## 1. INTRODUCTION

Edible mushrooms have been used for centuries as food and medicine. The yield of edible mushrooms has been increasing in recent years. According to the Turkish Statistical Institute, cultivated mushroom production in Türkiye in 2021 was 61 thousand tons (TÜİK, 2021). Thus, the unique taste, flavor, and huge nutritional value of mushrooms make them one of the most desired food items worldwide (Pérez-Chávez et al., 2019; Sun et al., 2020). These edible mushrooms are an important source of biologically active substances exhibiting beneficial (e.g., antioxidant, antitumor, antimicrobial, anti-inflammatory) effects on the human body (Baktemur et al., 2022b). Mushrooms have low energy and fat content but high protein, carbohydrate, and fiber content. They are especially popular among vegans and vegetarians due to their balanced amino acid content (Öztürk and Eyiler Kaya, 2022).

Among the most popular edible mushrooms, they include white button (*Agaricus bisporus*), portobello, shiitake (*Lentinula edodes*), maitake (*Grifola frondosa*), oyster mushroom (*Pleurotus* sp.) and enoki (*Flammulina velutipes*). All these mushrooms are produced using a substrate made of a wide variety of lignocellulosic waste, such as agricultural left-over residues (Pérez-Chávez et al., 2019). The valorization of lignocellulosic wastes is greatly enhanced through mushroom production. Some lignocellulosic materials, such as straw and sawdust used as mushroom substrate, are rich in lignin, hemicellulose and cellulose. However, depending on the mushroom species, specific types of substrate are required for high yielding of mushroom fruiting bodies (Atila, 2019a). The leftover of biomass or waste generated after mushroom production is known as "spent mushroom substrate (SMS)" or "spent mushroom compost" (SMC). Most of the time, it finds a second utility after usage (Phan and Sabaratnam, 2012; Pekşen and

Yamaç, 2016). Mushroom production generates a huge volume of SMS every year, causing a significant disposal challenge. Nowadays, SMS is becoming a useful resource for the generation of biogas, thus becoming fully integrated into the virtuous circle of resource reutilization for energy generation (Pérez-Chávez et al., 2019). Furthermore, the reuse of agricultural residues is part of the circular model for achieving a sustainable agricultural system as opposed to the linear model, which produces massive waste. All materials in the circular system attain their optimum level of utilization by recycling, and no waste is generated (Najari et al., 2022).

The primary concern arising from the search for better ways to manage agricultural waste for environmental sustainability prompted scientific communities to consider solutions for the present and future. As a result, the use of clean technology through integrated waste utilization is being promoted more than ever (Dey et al., 2021). In Türkiye, the huge volume of agricultural waste previously unutilized has become green gold and raw materials for mushroom production. Furthermore, recent studies conducted by Turkish researchers have revealed a huge potential of a wide range of agricultural wastes for the cultivation of high-value mushrooms such as *Ganoderma carnosum* Pat., reishi (*Ganoderma lucidum*), maitake (*Grifola frondosa*), and shiitake (*L. edodes*). This chapter portrays and comprehensively discusses the current status and potential use of agricultural residues for substrate preparation as well as the recycling of generated SMS for a sustainable agricultural system.

## **2. MUSHROOM PRODUCTION IN TÜRKİYE AND POPULAR SPECIES**

Türkiye has seen a dramatic increase in interest in mushroom production and consumption, as well as changes in eating habits, over the

last few decades. Mushrooms are considered part of a healthy and balanced diet due to their high levels of protein, fiber, carbohydrates, minerals, vitamins, and bioactive components. It was reported that between the years 2004-2018, while the commercial scale production of mushrooms increased in Türkiye, there was at the same time a significant increase in mushroom consumption, with 177% on average. Unfortunately, consumption per capita was still low compared to developed countries during the same period. It was around 579.2 g compared to 2.5 kg. Similar to other parts of the world, the most cultivated mushroom species are the white button mushroom (*A. bisporus*), oyster mushroom (*Pleurotus* spp.) and shiitake (*Lentinula edodes*) (Yıldız Turp and Boylu, 2018). The projection for future mushroom production in Türkiye was estimated using a statistical model (ARIMA, Box-Jenkins) by Kurt and Karayılmazlar (2019) based on data from previous years (1985–2016). It is expected that production will continue to rise and will exceed 100,000 tons by 2025.

### **3. LITERATURE ON MUSHROOM SUBSTRATE OPTIMIZATION IN TÜRKİYE**

In recent years, several Turkish researchers have embarked on the great cause of finding and optimizing mushroom substrates, mainly for high yield and agricultural sustainability. For instance, the following diversified raw materials have been examined in some key studies: corncob, grape pomace, green walnut husk, oak sawdust, peanut shell, poplar sawdust, sunflower meal, vine pruning waste, wheat bran, wheat stalk, wheat straw, and other materials (Atıla, 2019a; Baktemur et al., 2020; Aydın et al., 2021; Hal et al., 2021; Kara et al., 2021; Baktemur et al., 2022a; Baktemur et al., 2022b). Table 1 summarizes the some studies on mushroom substrate optimization in Turkey.

**Table 1:** Some studies on mushroom substrate optimization in Türkiye

N°	Mushroom specie	Mushroom substrate composition	Findings	References
1.	<i>Pleurotus species</i>	Hazelnut husk, wheat straw, wheat bran were used at different ratios.	Biological efficiency was found lower in the growing mixtures including hazelnut husk than the control (wheat straw+5% wheat bran).	(Peksen and Kucukomuzlu, 2004)
2.	<i>L. edodes</i>	Hazelnut husk was used alone and mixed with wheat straw, beech wood-chip and wheat bran at different ratios.	Hazelnut husk was proposed as basal substrate material in shiitake mushroom cultivation.	(Özçelik and Pekşen, 2007)
3.	<i>G. lucidum</i>	Tea waste was used.	The highest yield and biological efficiency were observed in 80 sawdust:20 tea waste.	(Peksen and Yakupoglu, 2008)
4.	<i>P. eryngii</i>	Wheat straw, soybean straw, corn stalk, bean stalk, millet straw, cotton stalk and rice barn were tested at different ratios.	The fastest spawn running was found in the growing mixture including wheat straw-bean stalk (1:1) and rice bran. The highest yield and biological efficiency were obtained from including wheat straw- millet straw and rice bran.	(Kirbag and Akyuz, 2008a)
5.	<i>P. eryngii</i> var . <i>ferulae</i>	Wheat straw, cotton straw, lentil straw and rice bran were used at different ratios.	The fastest spawn running, the highest yield and biological efficiency were determined in the growing mixture including wheat straw-cotton straw (1:1) and 20% rice bran.	(Kirbag and Akyuz, 2008b)
6.	<i>Lentinus tigrinus</i>	Poplar sawdust, oak sawdust, hornbeam	Mycelia development and yield were	(Peksen et al., 2009)

		sawdust, ash, wheat bran were used at the various levels.	favorable in oak sawdust, hornbeam sawdust and ash including 5-10% wheat bran.	
7.	<i>Pleurotus djamor</i> <i>Pleurotus citrinopileatus</i> <i>Pleurotus eryngii</i>	Wheat bran was mixed with oak sawdust, bean straw, safflower hay, sunflower head residue	The best results for mycelia development were obtained from safflower hay in all <i>Pleurotus</i> species. While biological efficiency was higher in safflower hay and bean straw than oak sawdust for <i>P. djamor</i> and <i>P. eryngii</i> , it was higher in oak sawdust for <i>P. citrinopileatus</i>	(Atila, 2017a)
8.	<i>P. ostreatus</i>	Chickpea straw was mix with cotton seed hulls, olive press cake, sunflower seed press cake, sugar beet pulp	The highest yield and biological efficiency were observed in the mix of chickpea straw:cotton seed hulls. The fastest spawn running was determined in chickpea straw.	(Atila, 2017b)
9.	<i>P. eryngii</i>	Poplar sawdust was mixed with additional raw materials such as sunflower meal, grape pomace and green walnut husk in different proportions.	High yield of <i>Pleurotus eryngii</i> were observed with the mix of poplar sawdust and sunflower meal. Additionally, they observed a superior spawn running time and cultivation period length when they used a mix of poplar sawdust and green walnut husk.	(Atila, 2019a)
10.	<i>Hericium erinaceus</i> <i>L. edodes</i>	Oak sawdust was mixed with grape pomace, green walnut hull, olive press cake, tea waste	The shortest spawn running time and the highest yield were obtained from the mix including grape	(Atila, 2019b)

			pomace for both <i>H. erinaceus</i> and <i>L. edodes</i> .	
11.	<i>L. edodes</i>	Oak sawdust, chickpea stalk, alfalfa hay and sunflower head residue were tested.	Maximum productivity was recorded in sunflower head residue. Substrate requirement of shiitake mushroom was determined as “moderate N amount, hemicellulose-lignin and low cellulose:lignin ratio”.	(Atila, 2019c)
12.	Shiitake ( <i>L. edodes</i> )	Wheat bran was mixed with additional raw materials such as oak sawdust, poplar sawdust, corncob, wheat stalk, peanut shell and vine pruning waste in different proportions.	The volatile aroma composition is significantly affected by substrate type. For instance, the use of corncob and wood straw affect considerably aldehydes and eight-carbon components content respectively in shiitake mushroom.	(Baktemur et al., 2020)
13.	<i>Hypsizygus ulmarius</i>	Bean straw, corn silage and wheat straw, poplar sawdust, pine sawdust and spent mushroom substrate were tested.	Bean straw was found to be the most suitable material in terms of spawn running time, yield and biological efficiency. Substrate requirement of shiitake mushroom was determined as “moderate N amount, hemicellulose-lignin and low lignin and high cellulose”.	(Öztürk and Atila, 2021)
14.	Maitake ( <i>G. frondosa</i> )	Wheat bran was mixed with additional raw materials such as oak sawdust, wheat stalk, and poplar sawdust were used in	Yield was obtained from only two substrate mixtures; oak sawdust + wheat stalk + wheat bran (1:1:1) and wheat stalk + wheat bran	(Aydın et al., 2021)

		different proportions.	(1:1). Additionally, among volatile aroma compounds, ketones were the most abundant.	
15.	<i>Reishi (G. lucidum)</i>	Wheat bran was mixed with additional raw materials such as oak sawdust, corncob, peanut shell, and vine pruning waste in different proportions.	The highest yield and biological efficiency was obtained with the substrate mix of corncob + wheat bran (2:1). The fastest mycelia development was observed with the substrate mix of oak sawdust + peanut shell + wheat bran (1:1:1) and vine pruning waste + wheat bran (2:1).	(Hal et al., 2021)
16.	<i>Maitake (G. frondosa)</i>	Wheat bran was mixed with additional raw materials such as oak sawdust, poplar sawdust and wheat stalk in different proportions.	The substrate mix of wheat stalk + wheat bran (1:1) gave a highest protein content. The yield could obtain with the substrate mix of oak sawdust + wheat stalk + wheat bran (1:1:1) and wheat stalk + wheat bran (1:1).	(Kara et al., 2021)
17.	<i>P. eryngii</i>	Bean pods, chickpea pods, lentil straw and barley straw were tested.	The shortest spawn running time was found in bean pods + chickpea pods (1:1).	(Akyüz and Kırbağ, 2022)
18.	<i>G. carnosum</i>	Several raw materials, including oak sawdust, peanut shell, and corncob, are mixed with wheat bran in various proportions.	High yield was obtained with the substrate mix of peanut shell + wheat bran (2:1). The fastest spawn running was detected with the substrates mix of corncob + wheat bran (2:1).	(Baktemur et al., 2022a)
19.	<i>L. edodes</i>	Wheat bran was mixed with	High yield was obtained with the	(Baktemur et al., 2022b)

		additional raw materials such as oak sawdust, poplar sawdust, wheat stalk, peanut shell, corncob and vine pruning waste in different proportions.	substrate mixture of oak sawdust + wheat stalk + wheat bran (1:1:1). High biological efficiency was detected in the mix of wheat stalk + wheat bran (3:1) and oak sawdust + wheat stalk + wheat bran (1:1:1). The fastest spawn running was determined in the mix of corncob + wheat bran (3:1).	
20.	<i>Pholiota nameko</i>	Wheat bran was mixed with additional raw materials such as oak sawdust, peanut shell, almond shell and wheat stalk in different proportions.	High yield and biological efficiency was obtained with the substrate mixture of peanut shell + wheat bran (2:1). The fastest spawn running was determined in the mix of oak sawdust and wheat bran (2:1).	(Daşdelen, 2022)
21.	<i>P. eryngii</i>	Oak sawdust, poplar sawdust, wheat stalk, peanut shell, corncob, vine pruning waste and heat bran were mixed at different ratios.	The fastest spawn running time, the highest yield and biological efficiency were observed in the mix of peanut shell and wheat barn (2:1) and peanut shell, oak sawdust and wheat barn (1:1:1).	(Baştuğ et al. 2022)

As illustrated in Table 1, substrates and mushroom species have a significant impact on yield and quality parameters, particularly mushroom fruiting body size. Atila (2019) has made similar observations. For substrate preparation, different agricultural waste materials (peanut shell, poplar sawdust, corncob, wheat stalk, and vine pruning waste) are cautiously

selected by proper volume percentages (Figure 1). In most cases, the main ingredient, wheat bran, is supplemented into the ready mixture at approximately 5%. Afterward, the mixture is moistened at 70% humidity and kept in appropriate containers, during which the pH is adjusted up to 6-7 using lime. After these steps, substrate mixtures are filled into 1 kg high temperature resistant polypropylene bags and ready for sterilization in an autoclave (at 121 °C under 1.2 atm pressure for 90 minutes). At this stage and after sterilization, the substrate mixture is ready for spawn (Baktemur et al., 2020; Aydın et al., 2021). The substrate preparation in research institutions often differs from industrial production seen in big production companies all over Turkey, where the substrate mixture from various agricultural waste can begin at a minimum volume of 1 ton and beyond. This means that big machineries are needed for selecting, mixing, pre-soaking for appropriate humidity and for other processes till obtaining mushroom fruit bodies ready for packing (Baktemur, 2021).



**Figure 1:** Substrate composition and preparation.

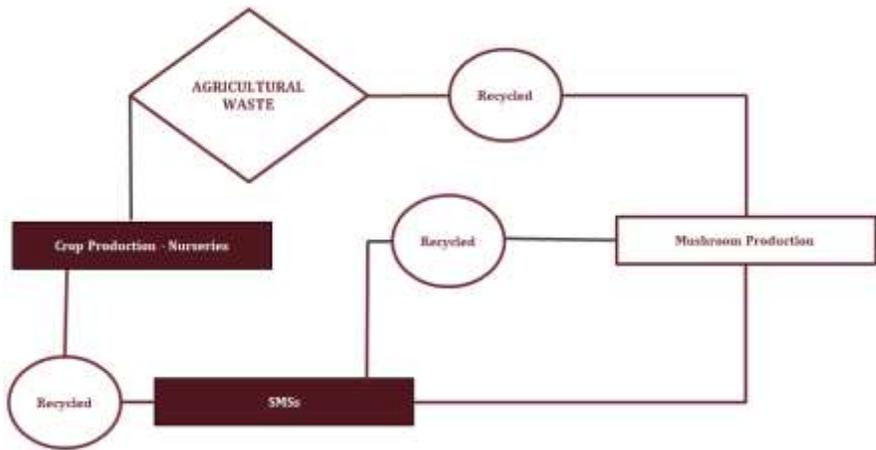
- (A) Wheat bran mixed with additional raw materials such as corncob
- (B) Wheat bran mixed with additional raw materials such as soy flour and poplar sawdust.
- (C) Wheat bran mixed with additional raw materials such as soy flour and wheat stalk.

#### 4. EFFECT OF DIFFERENT SUBSTATES ON THE AROMA COMPOSITIONS OF MUSHROOMS

Aroma is one of the most important factors influencing consumer decisions. Many factors have been reported to influence the aroma of edible mushrooms, including species, cultivation conditions, maturity, grading, mushroom parts, and processing and storage methods. Volatile compounds are primarily responsible for the distinctive aromas of mushrooms. Mushrooms are believed to synthesize natural aromatic volatile compounds for defense or as products of secondary metabolism (Sun et al., 2020). Key volatile compounds in mushrooms include aldehydes, hydrocarbons, ketones and esters (Aydın et al., 2021). For instance, when Baktemur et al. (2020) evaluated the changes in the aroma profile of shiitake mushrooms in various substrates composed of different proportions of poplar sawdust, oak sawdust, peanut shell, wheat stalk, corncob, and vine pruning waste mixed with wheat bran, the overall volatile aroma composition was significantly affected. Dimethyl tri-sulfide, benzaldehyde, dimethyl disulfide, 1-octen-3-ol, and 3-octanone were the major volatile components observed during the experiment. Thus, "1-octen-3-ol," also known as mushroom alcohol, and the eight-carbon compound "3-octanone" are both aliphatic alcohols and key aromas that give that particular mushroom-like scent (Taşkın et al., 2019). It is reported that in maitake (*Grifola frondosa*) and shiitake (*Lentinula edodes*), 3-octanone compound is often found in high levels when the used substrate incorporates some level of oak sawdust in substrate mixtures (Baktemur et al., 2020; Aydın et al., 2021).

## **5. RECYCLING OF THE SPENT MUSHROOM SUBSTRATE SMS**

In a mushroom farm, the biggest challenge is the disposal management of the huge volume of generated SMS. However, a significant portion of SMS is used as animal feedstock and fertilizer (Mohd Hanafi et al., 2018). In this era of the circular economy, SMSs have demonstrated immense potential in a wide range of applications. SMSs are degraded after the collection of mushroom fruit bodies. Depending on mushroom species, the degradation efficiency is estimated to be 40–80%. Furthermore, it is reported that the composition of SMS varies greatly depending on location, type of mushroom grown, and other factors (Leong et al., 2022). According to Pekşen and Yamaç (2016), approximately 170-250 thousand tons of mushrooms are produced in Turkey each year, with approximately 3-5 kg of SMS produced for every kg of mushroom produced. SMS material is known to contain microorganisms, fungal mycelium, and a wide variety of lignocellulosic enzymes depending on the initial grown mushroom species. Those enzymes are useful in biotechnological processes and include lignin peroxidase, laccase, manganese peroxidase, versatile peroxidase, xylanase and cellulose and others (Pekşen and Yamaç, 2016; Yamaç and Pekşen, 2016). In the production of edible mushrooms, raw materials such as wheat straw, paddy straw, maize cob, bran, sawdust, livestock litter, rice straw, hazelnut husk, cottonseed meal are recycled from SMS and re-used again in agricultural production. Many industries benefit from SMS as a valuable byproduct due to its high organic matter content and nutrients like P and K. Before their re-use, they are often pre-washed and mixed with other materials such as soil and peat, mainly for crop seedling production (See Figure 2.) (Pekşen and Yamaç, 2016).



**Figure 2:** Process flow of agricultural waste and SMS valorization in Turkey.

Turkish researchers have demonstrated that SMS from *A. bisporus* and *Pleurotus ostreatus* can be mixed with peat for tomato seedling growth and development (Yılmaz et al., 2018). Additionally, SMS can be employed as a cover material for lawn grass production. For instance, it was found that a mixture of soil and SMS (1:1, w/w) was more effective (Doyran et al., 2018). SMS constitutes an alternative to the peat and perlite (3:1, w/w) mixture consistently employed for pepper seedling growth and development. For instance, Demir (2017) demonstrated that the mixture of SMS and perlite (3:1, w/w) can be as effective as the mixture of peat and perlite for the growth of Charleston pepper seedlings.

## 6. CONCLUSION AND PERSPECTIVES

There is an advancement in the valorization of agricultural waste in Türkiye, particularly in mushroom production. Various studies have demonstrated different substrate mixtures can be advantageous in growing new cultivated mushroom species in the country, like *Pholiota nameko* and maitake (*G. frondosa*). In this chapter, we also explore the reuse or recycling of mushroom substrate after mushroom harvesting. The leftover material,

known as SMS, is beneficial to numerous industries and can be employed again for mushroom production after thorough processing. Additionally, the same material is employed in vegetable nurseries for pepper and tomato growth and development. Nevertheless, more research and more involvement of mushroom producers are needed to completely achieve the circular model of a sustainable agricultural system where all agricultural waste will be recycled. We are hoping that substrate optimization studies will lead to large scale industrial applications.

## REFERENCES

- Akyüz, M. & Kırbag, S. (2022). Cultivation of king eryngii (*Pleurotus eryngii* (DC. ex Fr.) Quel.) isolates on various local agro-residues. *Biomass Conv. Bioref.* <https://doi.org/10.1007/s13399-022-03051-6>
- Atila, F. (2017). Evaluation of Suitability of Various Agro-Wastes for Productivity of *Pleurotus djamor*, *Pleurotus citrinopileatus* and *Pleurotus eryngii* Mushrooms. *Journal of Experimental Agriculture International*, 17(5), 1-11.
- Atila, F. (2017). Biodegradation of Different Agro-Industrial Wastes through the Cultivation of *Pleurotus ostreatus* (Jacq. ex Fr) Kummer. *Journal of Biological and Environmental Sciences*, (31), 11-12.
- Atila, F. (2019a). Yield and Fruit Body Properties of *Pleurotus eryngii* Isolates Grown on Poplar Sawdust Supplemented with Different Additive Materials. *Mantar Dergisi*, 2nd International Eurasian Mycology Congress 2019 ve XI. Türkiye Yemeklik Mantar Kongresi-2019 Özel Sayısı, 106-113. Retrieved from <https://dergipark.org.tr/en/pub/mantar/issue/50932/619151>
- Atila, F. (2019b). The Use of Phenolic-rich Agricultural Wastes for *Hericium erinaceus* and *Lentinula edodes* Cultivation. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 56 (4), 417-425.
- Atila, F. (2019c). Compositional changes in lignocellulosic content of some agro-wastes during the production cycle of shiitake mushroom. *Scientia Horticulturae*, 245, 263-268. <https://doi.org/10.1016/j.scienta.2018.10.029>
- Aydin, M. Z., Süfer, Ö., Baktemur, G., Shimira, F., & Taşkın, H. (2021). Effect of Different Substrate Mixtures on Volatile Aroma Compounds and Antioxidant Activity of Maitake (*Grifola frondosa*) Mushroom. *Turkish Journal of Agriculture-Food Science and Technology*, 9(6), 1037-1046. <https://doi.org/10.24925/turjaf.v9i6.1037-1046.4116>
- Baktemur, G. (2021). Beyaz Şapkalı Mantar (*Agaricus bisporus*) Yetiştiriciliği. *Akademisyen Kitabevi A.Ş.* Ankara, Turkey.
- Baktemur, G., Çelik, Z. D., Kara, E., & Taşkın, H. (2020). The effect of different agricultural wastes on aroma composition of shiitake (*Lentinula edodes* (berk.) pegler) mushroom. *Turkish Journal of Agriculture-Food Science and Technology*, 8(7), 1540-1547. <https://doi.org/10.24925/turjaf.v8i7.1540-1547.3415>
- Baktemur, G., Kara, E., Yazar, M., Soylu, M. K., & Taşkın, H. (2022a). Use of different agricultural wastes in *Ganoderma carnosum* Pat. cultivation. *Turkish Journal of Agriculture and Forestry*, 46(3), 352-358. <https://doi.org/10.55730/1300-011X.3008>
- Baktemur, G., Kara, E., Yazar, M., Yılmaz, N., Ağçam, E., Akyıldız, A., & Taşkın, H. (2022b). Yield, quality and enzyme activity of shiitake mushroom (*Lentinula edodes*) grown on different agricultural wastes. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 50(1). <https://doi.org/10.15835/nbha50112553>
- Baştuğ, G., Hal, Y.B., Baktemur, G., Yazar, M., Kara, E., & Taşkın H. (2022). Effect of growing mixtures including different agricultural wastes on yield and quality of *Pleurotus eryngii*. *Mustafa Kemal University Journal of*

- Agricultural Sciences, <https://doi.org/10.37908/mkutbd.1098660.1098660> (accepted)
- Daşdelen, O. (2022). Effects of different agricultural wastes on yield and quality in *Pholiota nameko* cultivation. MSc Thesis, Institute of Natural and Applied Sciences, Cukurova University, Adana, Turkey.
- Demir, H. (2017). The effects of spent mushroom compost on growth and nutrient contents of pepper seedlings. *Mediterranean Agricultural Sciences*, 30 (2), 91-96.
- Dey, T., Bhattacharjee, T., Nag, P., Ghati, A., & Kuila, A. (2021). Valorization of agro-waste into value added products for sustainable development. *Bioresource Technology Reports*, 16, 100834. <https://doi.org/10.1016/j.biteb.2021.100834>
- Doyran, Y. E., Özkutlu, F., Akgün, M. & Ete, Ö. (2018). Use of Mushroom Compost as Covering Material for Lawn Areas. *Ordu University Journal of Science and Technology*, 8 (1), 38-49.
- Hal, Y. B., Yarar, M., Kara, E., Baktemur, G. & Taşkın, H. (2021). Effect of Different Agricultural Wastes on Yield and Quality in Cultivation of *Ganoderma lucidum* (Reishi mushroom). *Çukurova J. Agric. Food Sci.*, 36 (2), 275-288. <https://doi.org/10.36846/CJAFS.2021.55>
- Kara, E., Baktemur, G., Yarar, M. & Taşkın, H. (2021). Effect of different growing substrates on yield and quality in cultivation of maitake (*Grifola frondosa*) mushroom. *Akademik Ziraat Dergisi*, 10 (2), 201-218. <https://doi.org/10.29278/azd.831748>
- Kirbag, S. & Akyuz, M. (2008a). Effect of various agro-residues on growing periods, yield and biological efficiency of *Pleurotus eryngii*. *Journal of Food, Agriculture & Environment*, 6 (3&4), 402-405.
- Kirbag, S. & Akyuz, M. (2008b). Evaluation of agricultural wastes for the cultivation of *Pleurotus eryngii* (DC. ex Fr.) Quel. var. *ferulae* Lanzi. *African Journal of Biotechnology*, 7 (20), 3660-3664. <https://doi.org/10.5897/AJB08.583>
- Kurt, R. & Karayılmazlar, S. (2019). Mushroom production and projection in Turkey using ARIMA (Box-Jenkins). *Turkish Journal of Forestry Research*, 6 (1), 72-76. <https://doi.org/10.17568/ogmoad.461534>
- Leong, Y. K., Ma, T. W., Chang, J. S., & Yang, F. C. (2022). Recent advances and future directions on the valorization of spent mushroom substrate (SMS): A review. *Bioresource Technology*, 344, 126157. <https://doi.org/10.1016/j.biortech.2021.126157>
- Mohd Hanafi, F. H., Rezanía, S., Mat Taib, S., Md Din, M. F., Yamauchi, M., Sakamoto, M., Hara, H., Park, J., & Ebrahimi, S. S. (2018). Environmentally sustainable applications of agro-based spent mushroom substrate (SMS): an overview. *Journal of Material Cycles and Waste Management*, 20(3), 1383-1396. <https://doi.org/10.1007/s10163-018-0739-0>
- Najari, Z., Khodaiyan, F., Yarmand, M. S., & Hosseini, S. S. (2022). Almond hulls waste valorization towards sustainable agricultural development: Production

- of pectin, phenolics, pullulan, and single cell protein. *Waste Management*, 141, 208-219. <https://doi.org/10.1016/j.wasman.2022.01.007>
- Özçelik., E., & Pekşen, A. (2007). Hazelnut husk as a substrate for the cultivation of shiitake mushroom (*Lentinula edodes*). *Bioresour Technology*, 98(14), 2652-8. <https://doi.org/10.1016/j.biortech.2006.09.020>
- Öztürk, C., & Atila, F. (2021). Changes in lignocellulosic fractions of growing substrates during the cultivation of *Hypsizygus ulmarius* mushroom and its effects on mushroom productivity. *Scientia Horticulturae*, 288. <https://doi.org/10.1016/j.scienta.2021.110403>
- Öztürk, N. & Eyiler Kaya, E. (2022). Nutritional values and health effects of popular mushrooms. *The Journal of Food*, 47 (4), 539-563. <https://doi.org/10.15237/gida.GD22027>
- Peksen, A. & Kucukomuzlu, B. (2008). Yield Potential and Quality of Some *Pleurotus* Species Grown in Substrates Containing Hazelnut Husk. *Pakistan Journal of Biological Sciences*, 7(5), 768-771. <https://doi.org/10.3923/pjbs.2004.768.771>
- Peksen, A. & Yakupoglu, G. (2008). Tea waste as a supplement for the cultivation of *Ganoderma lucidum*. *World Journal of Microbiology and Biotechnology*, 25, 611-618.
- Peksen, A., Yakupoglu, G. & Kibar, B. (2009). Influence of Various Sawdust-Based Substrates from Different Wood Species and Supplementary Wheat Bran on Yield And Fruiting Body Size of *Lentinus tigrinus*. *Acta Horticulturae*, 830, 319-326. <https://doi.org/10.17660/ActaHortic.2009.830.45>
- Pekşen, A. & Yamaç, M. (2016). Using Areas of Spent Mushroom Compost/Substrate - 1: Properties and Importance, *The Journal of Fungus*, 7 (1), 49-60. <https://doi.org/10.15318/Fungus.2016118354>
- Pérez-Chávez, A. M., Mayer, L., & Albertó, E. (2019). Mushroom cultivation and biogas production: A sustainable reuse of organic resources. *Energy for Sustainable Development*, 50, 50-60. <https://doi.org/10.1016/j.esd.2019.03.002>
- Phan, C. W., & Sabaratnam, V. (2012). Potential uses of spent mushroom substrate and its associated lignocellulosic enzymes. *Applied Microbiology and Biotechnology*, 96(4), 863-873. <https://doi.org/10.1007/s00253-012-4446-9>
- Sun, L. B., Zhang, Z. Y., Xin, G., Sun, B. X., Bao, X. J., Wei, Y. Y., Zhao, X. M. & Xu, H. R. (2020). Advances in umami taste and aroma of edible mushrooms. *Trends in Food Science & Technology*, 96, 176-187. <https://doi.org/10.1016/j.tifs.2019.12.018>
- Taşkın, H., Çelik, Z. D., Bozok, F., Cabaroğlu, T., & Büyükalaca, S. (2019). First Report on the Volatile Composition of *Tricholoma anatolicum* in Comparison with *Tricholoma caligatum*. *Records of Natural Products*, 13(6). <http://doi.org/10.25135/rnp.122.18.12.1095>
- TÜİK, 2021. Türkiye İstatistik Kurumu - Turkish Statistical Institute. Retrieved July 19, 2022 from: <http://www.tuik.gov.tr/Start.do>
- Yıldız Turp, G. & Boylu, M. (2018). Medicinal and Edible Mushrooms & Usage in Meat Products. *Yuzuncu Yil University Journal of Agricultural Sciences*, 28 (1), 144-153. <https://doi.org/10.29133/yyutbd.397683>

Yılmaz, C., Sırça, E., Özer, H. & Pekşen, A. (2018). Usage of *Agaricus* and *Pleurotus* Spent Mushroom Composts as Growth Medium in Tomato Seedling Production. *Turk J Agric Res*, 5(3), 229-235. <https://doi.org/10.19159/tutad.423773>



## **CHAPTER 11**

### **NEW APPROACHES in *Cyclamen* BREEDING**

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## **INTRODUCTION**

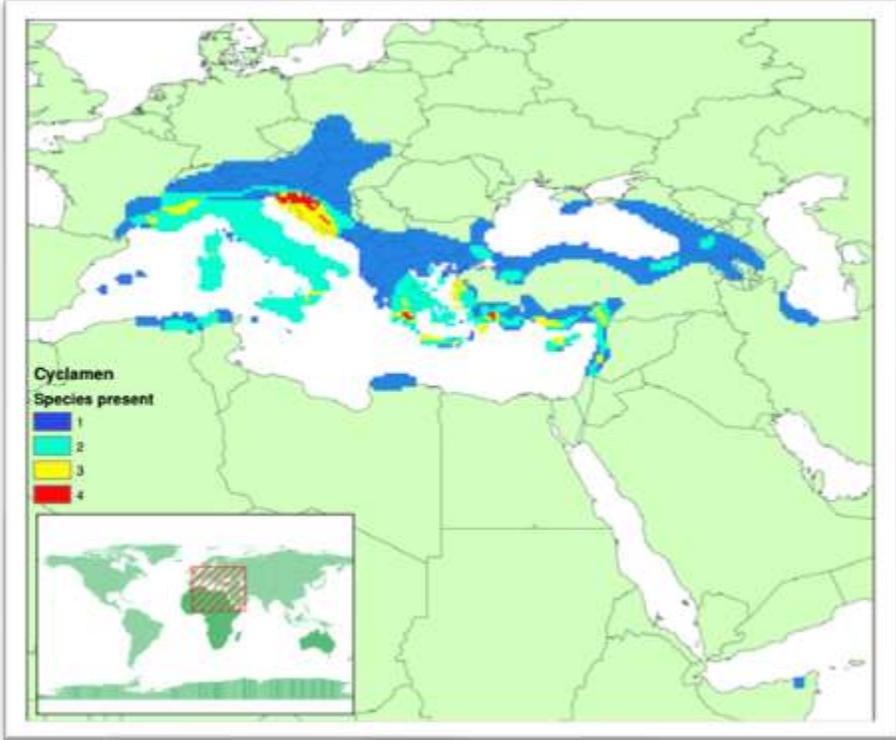
### **1. *Cyclamen***

#### **1.1. Origin and Taxonomy**

The name is *Cyclamen*, BC. It was given by Theophrastus, who lived between 370-285 BC. The word cyclamen, "kylos", means circle and is derived from the Latin words "cyclamnos". It is so named because the underground tubers of these plants are round, the leaves are in the shape of a circle, or the fruit's stalks extend into the soil by making spirals in the form of curves (Tanker ve Turkoz, 1984; Kocak, 2019). Europeans in the early 17<sup>th</sup> century collected natural cyclamens from Anatolia, the Middle East, and the Mediterranean basin, and brought them to Western Europe. Some species of cyclamen have been cultivated in Western European countries since the 18<sup>th</sup> century. Later, its economic importance increased and breeding work was started in *Cyclamen*. It became a popular commercial plant in the 19<sup>th</sup> century. The only commercially produced species is *Cyclamen persicum* Miller. (Prange et al. 2010; Amini, 2014; Erturk, 2022).

*Cyclamen* is one of the plant species that has been used as a potted ornamental plant and has commercial importance. (Motoyasu ve Takiko 1991; Winkelman et al. 2003; Seyring et al. 2009). Popular among perennial ornamental plants and economically important (Winkelman, 2010). Magnoliophyta (angiosperms) subdivision of the plant kingdom, the Magnoliopsida (dicots) class, is included in Ericales order (Curuk et al. 2021). *Cyclamen* originated from the Primulaceae family and is known to be included in the Myrsinaceae family by reclassification (Debussche et al. 2004; Curuk et al. 2015, 2016; Shafiei-Masouleh, 2022). About 23 species, six of which are endemic to, Turkey (Izgu et al. 2016; Cornea-Cipcigan et al. 2020). However, according to Compton et al, (2004) and Yesson and Culham (2006), there are 21 species. The highest cyclamen diversity is in Turkey with 11 species, followed by Greece (Culham et al. 2006; Yesson and Culham, 2011).

The origin of the cyclamen species is the Mediterranean basin and they spreads from Europe to the east of Iran and one species is found in Somalia (Curuk et al. 2015; Izgu et al. 2016; Mohammed et al. 2018). The *Cyclamen* genus in the world is given in Figure 1.



**Figure 1.** *Cyclamen* diversity (Yesson and Culham, 2006)

*Cyclamen* genus naturally spreads mostly in the Mediterranean and Black Sea regions in Adana, Mersin, Osmaniye, Çanakkale, Giresun, Antalya, Konya, Amasya, Artvin, Aydın, Balıkesir, Bolu, İzmir, Rize and Trabzon provinces. Flowering time varies by species, even among different species grown in the same location. The flowering seasons of cyclamen can be divided into two different groups as autumn and spring blooming (Debussche et al. 2004; Takamura, 2007; Curuk et al. 2015; Alp, 2020). Turkish flora *Cyclamens* are given in figure 2.

Accordingly, those that bloom in autumn are:

- *Cyclamen cilicium* Boiss. et Heldr.,
- *Cyclamen hederifolium* Aiton.,
- *Cyclamen mirabile* Hildebr.
- *Cyclamen graecum* Link.,

And those that bloom in the spring:

- *Cyclamen alpinum* Dammann ex Spreng,
- *Cyclamen persicum* Miller.,
- *Cyclamen pseudibericum* Hildebr.,
- *Cyclamen coum* Miller.,
- *Cyclamen parviflorum* Pobed.,
- *Cyclamen trochopteranthum* O. Schwarz are species (Gundogan, 2003).

The species endemic to our country are;

- *Cyclamen pseudibericum* Hildebr.,
- *Cyclamen trochopteranthum* O. Schwarz.
- *Cyclamen cilicium* Boiss. et Heldr.,
- *Cyclamen cilicium* var. *intaminatum* Meikle
- *Cyclamen parviflorum* Pobed.,
- *Cyclamen mirabile* Hildebr. known as types (Ekim et al., 1991; Takamura, 2007).

Since the tubers of cyclamen are dug out of the soil by pigs, they are called 'buckthorn, ground loaf, buckthorn, rabbit ear, mountain violet, cyclamen, frankincense, frankincense, Virgin Mary, etc.' Names such as violet root, calf's belly, prairie violet, mole, köstüköpen, kötüköpeği, couscous, rabbit's trotter' are given. In other countries, it is referred to as something like an English bread loaf because they are said to be eaten by pigs: in French, pain de pourceau, in Italian pan porcino, in Dutch varkensbrood, in French "pork manjū" in Japanese (Kaykıcı et al. 2012; Mohammed et al. 2018; Aydın et al. 2014; Curuk, 2013).

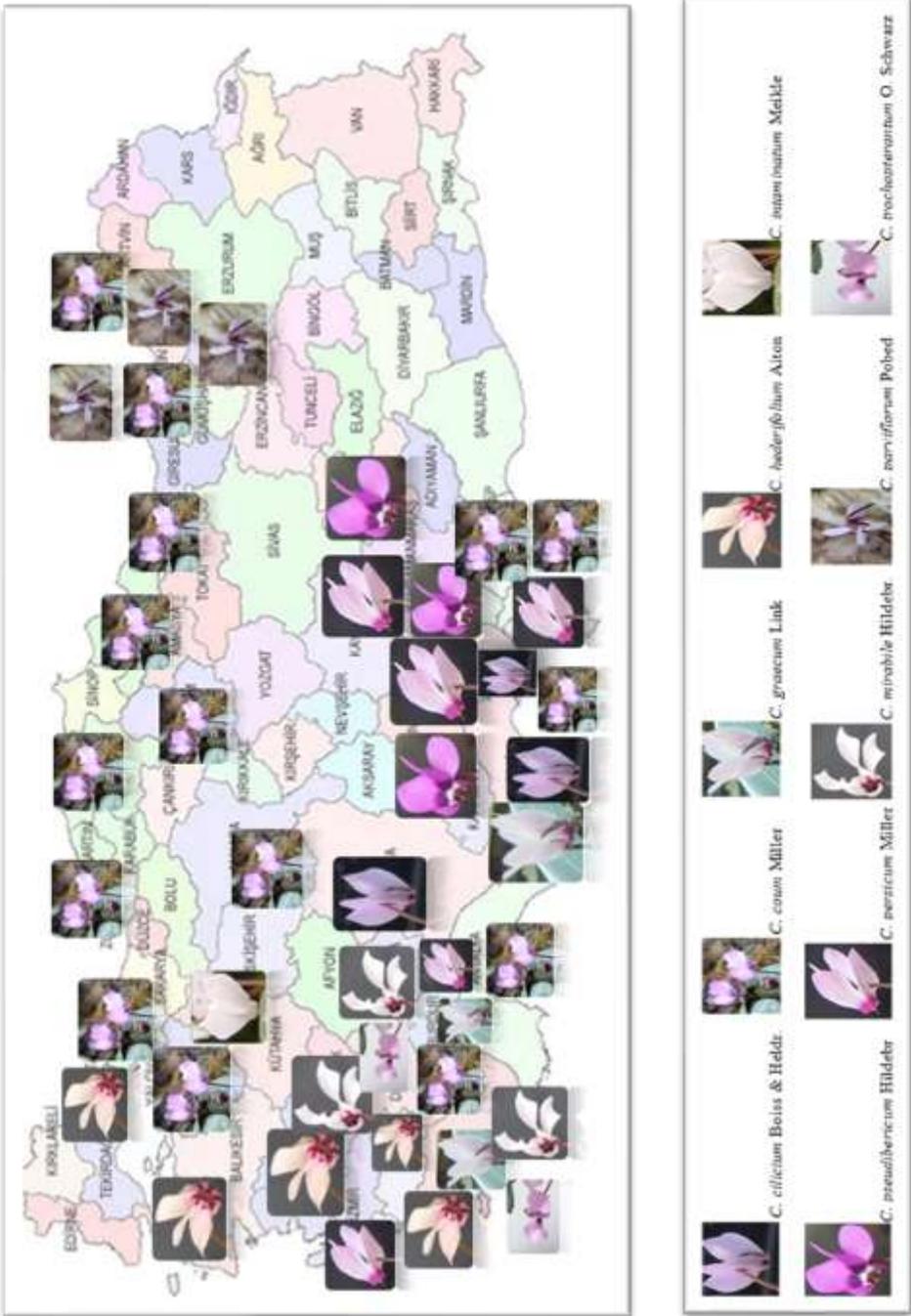


Figure 2. Turkish flora Cyclamens (Yalcın Mendi and Curuk, 2016)

## 1.2. Usage in Traditional Medicine

The use of the cyclamen plant was known to the Egyptians, Greeks and Romans (Mazouz and Djeddi, 2013). The use of cyclamen was widespread in the Middle Ages, and it is increasingly being used for its use in the treatment of rheumatic and arthritic (Mohammed et al. 2018). *Cyclamen* tubers collected in the spring are used for various ailments such as headaches and earaches, colds, toothaches, gas, digestive disorders, rheumatism and skin diseases (Mazouz and Djeddi, 2013). In addition, cyclamen is also used as soap (Yaldız et al. 2010).

Saponins are commercially important compounds and are used in food, cosmetics, pharmaceutical industry anticancer, antioxidant, and antihypertensive (Mammadov, 2014). It is generally believed that plants with higher saponin content show good cytotoxic activity, i.e, there is a direct correlation between total saponin content and cytotoxic activity. There are some studies on the chemical composition of several *Cyclamen* species, containing some triterpene saponins and glycosides. (Altunkeyik et al. 2012). *Cyclamen* tubers contain abundant toxic saponins. (Zeybek and Zeybek, 1994). In addition, saponosity, a substance obtained from tubers, is called cyclamine, and it has been found that saponizide reduces blood cholesterol levels and has antitumoral and antimicrobial effects. Again, it is reported that saponizides in these tubers are diuretic, anti-exudative and effective in tinnitus (Yaldız et al. 2010). Roots contain triterpene glycosides known as saponins (Mohammed et al. 2018). In addition, analgesic, anti-inflammatory and antimicrobial activities of some *Cyclamen* species, such as *C. repandum* and *C. mirabile* have been reported (Dall'acqua et al. 2010). *Cyclamen repandum* is widely used in traditional medicine for reasons such as laxatives and abortions. (Speroni et al. 2007). Also, the tuber *Cyclamen hederifolium* Aiton is used against warts (Pieroni et al. 2004). *Cyclamen coum* is used in the

treatment of boils, spots and sunburns, gout, visual impairment, jaundice and poisonous animal bites (Yaldız et al. 2010). *Cyclamen coum* var. the tubers of the coum plant are used in the treatment of infertility (Calis et al. 1997).

### 1.3. Plant Morphology

*Cyclamen*, with its elegant flowers and showy leaves, is a geophyte that grows in the shade of trees and bushes in temperate regions and its natural species in forest areas. It is the most famous and has an important share in trade in many countries and is used as an indoor (potted) and/or outdoor plant (Takamura, 2007). In addition, Winkelmann et al. (2010), in their report; reported that cyclamen was used as a cut flower. Grey-Wilson (2015), in the Netherlands and Germany, a large amount of cultivation is carried out for this purpose. *Cyclamens* with showy flowers have a vase life of more than 12 days and even last up to 3 weeks (Neumaier et al. 2009). Because the flowers of *Cyclamen purpurascens* have a sweet scent like lily of the valley, they are a valuable gene source for improving the scent of flowers in cyclamen breeding (Ishizaka and Uematsu, 1995). *Cyclamen hederifolium* is used as a garden plant, but its importance is very limited (Takamura, 2007). *Cyclamen coum* species has an important potential as an outdoor ornamental plant because it is resistant to cold (Sevindik, 2018). *Cyclamen persicum*, on the other hand, is a commercially grown species and has larger and taller flowers than other species, so it is highly preferred in commercial production. It grows in the height of 5-20 cm and blooms from autumn to late spring. A plant develops approximately 20-30 flower stalks (60-80 in newly developed cultivars), and produces between 20 and 70 seeds, depending on the

variety, in a fruit capsule that occurs at the tip of the stalk. The plant can be propagated by seed as well as by dividing the tuber (Curuk et al. 2021). The general view of Wild Cyclamen is given in Figure 3.

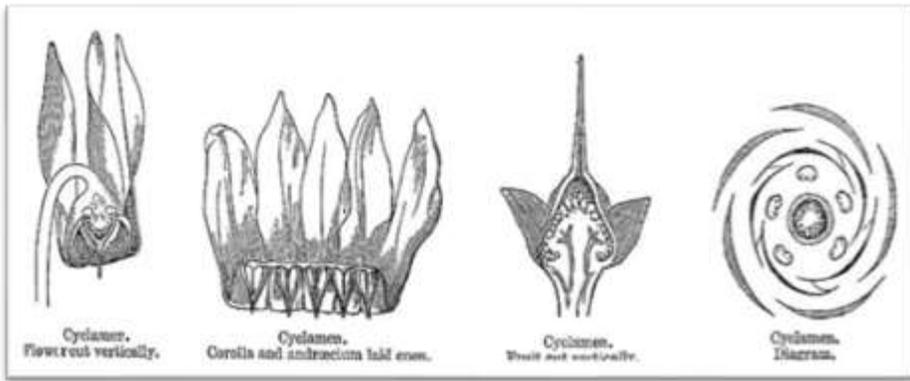


**Figure 3.** General view of wild *Cyclamen* (Original: Tansu Hastürk)

#### **1.4. Flower Morphology and Biology**

The flowers of the cyclamen plant differ from the flowers of many other plant species in that their petals are curved upwards. The development of the flower stalk (pedicel) on the tuber is the same as that of the leaves. The tip of the flower bud always points down, and the five petals curl upwards when the bud opens. Petals can be in shades of pink, purplish red, red, or white with a distinctive mark or spot at the base. Petals differ according to the species. For

example, it can be narrow, elliptical, or rounded, or broadly bent. The open space containing the style and stamen is pentagonal when viewed from below. In *Cyclamen*, the stigma is pollinated between species as it matures before the pollen is ready. Pollination usually takes place with some types of insects or bees. There is also some evidence that self-pollination is possible. Touching the flower when the pollen is fully ripe will likely drop a lot of pollen stuck on the stigma (Curuk et al. 2021). The flower structure of the *Cyclamen* genus is given in figure 4.



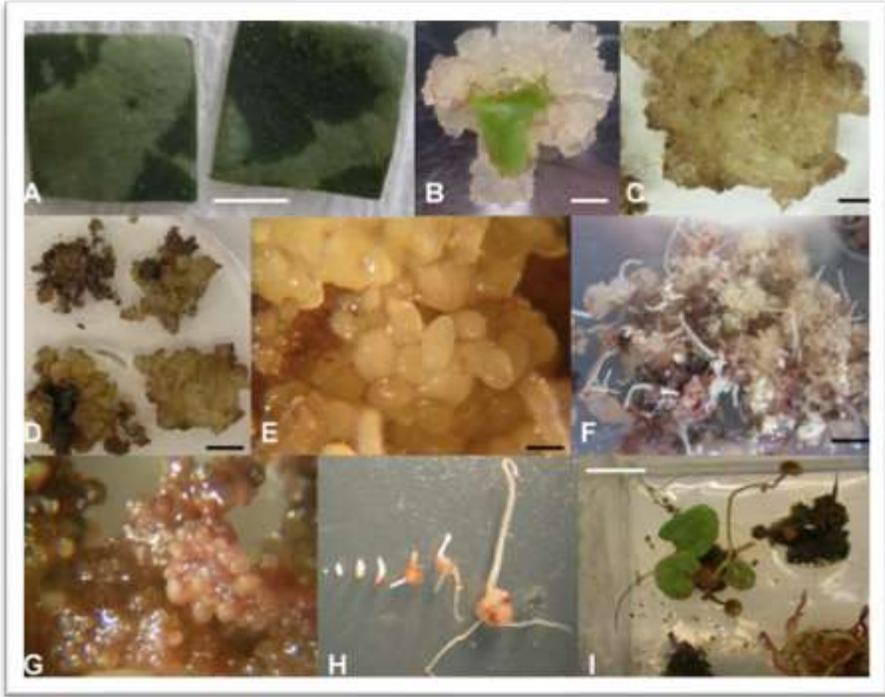
**Figure 4.** Flower structure of *Cyclamen* plant (Metin, 2012)

### 1.5. Production Methods

The cyclamen plant reproduces by seed in its natural habitat. Although the number of flowers in a plant in the genus *Cyclamen* varies between 20-30 on average, an average of 20 to 70 seeds are obtained from each flower (Curuk et al. 2021). The commercial cultivation of the cyclamen plant is done by seed. It is propagated by the traditional method of crossbreeding, that is, by the seeds produced by the manual crossing process one by one and therefore expensive. In addition, this process is quite laborious and takes a long time (Wellensiek, 1959). *Cyclamen* is propagated by seed, although it has disadvantages, such as the high cost of producing F1-hybrid seeds (Tagipur et al. 2016). Winkelmann (2010), reported that cyclamen cannot be propagated

by cuttings or tuber division, and therefore would be propagated by *in vitro* techniques. Vegetative propagation by tissue culture is an effective propagation method in cyclamen. Recently, propagation of a wide variety of ornamental plants by tissue culture has become an accepted commercial practice and rapid steps have been taken in the field of micropropagation of ornamental plants (Read ve Preece, 2007; Jalali et al. 2012). There are protocols for micropropagation of cyclamen by organogenesis and somatic embryogenesis (Dillen et al. 1996; Winkelman, 2010).

Many researchers began to study *cyclamen* after Mayer (1956) reported on the *in vitro* propagation of cyclamen through organogenesis (Takamura, 2007). Okumoto and Takabayashi (1969), reported that success was achieved by culturing *cyclamen* tuber explants *in vitro* and could be used as a vegetative propagation method in tissue culture. It has been reported to regenerate from *cyclamen*, petiole, petal, peduncle, cotyledon, leaf, tuber and root explants (Mayer, 1956; Lowenberg, 1969; Wicart et al. 1984). In addition to other explants, ovules and ovaries are used in the regeneration of *cyclamens* (Izgu, et al.; Kocak et al. 2014). There are two main methods of cyclamen regeneration by tissue culture, organogenesis and somatic embryogenesis (Takamura, 2007). The development of *Cyclamen* genus *in vitro* is given in figure 5. In addition to studies of organogenesis in *Cyclamen* (Dillen et al. 1996; Takamura & Miyajima, 1997; Murasaki and Tsurushima 1988; Schwenkel and Grunewaldt 1988), there are effective protocols for somatic embryogenesis (Winkelman and Serek 2005; Winkelman 2010; Kocak et al. 2014; Izgu et al. 2016).



**Figure 5.** Developmental stages of somatic embryogenesis in cyclamen. (A) Leaf explant; (B, C, D) Callus from leaf explant after 4, 8 and 12 weeks of culture, respectively; (E–G) Somatic embryos in plant growth regulator (PGR)-free medium after 10 weeks (H) Typical developmental stages of somatic embryos 10 weeks after transfer into PGR-free medium; (I) Plants regenerated from somatic embryos (Kocak et al. 2014)

## 2. Breeding Purposes and Methods

### 2.1. Breeding Purposes

The history of most ornamental plants began in the 17<sup>th</sup> and 18<sup>th</sup> centuries, when botanists, plant collectors, missionaries, and ship doctors brought new plants to Europe from the East and the New World (Horn, 2004). At the beginning of breeding in ornamental plants, gardeners and curious growers observed an increase in height and development of plants by crossing ornamental plants. In the early 18<sup>th</sup> century, more conscious cross-breeding studies began. (Horn, 2002; Balkaya et al. 2021). The main goals in many

commercial ornamental breeding programs remain unchanged: selecting new varieties with improved flower characteristics (color, shape, fragrance, improved vase life), leaf characteristics and plant habitus, as well as increasing global consumer concern for the environment, awareness of the effects of climate change, and natural Growers pushed by the trend towards sustainable products and production methods are forced into innovative breeding goals (Van Huylenbroeck, 2020).

*Cyclamen persicum* was brought to Western Europe in the 17<sup>th</sup> century and gradually became popular in the mid-19<sup>th</sup> century (Doorenbos, 1950). Almost all cyclamen cultivars have been bred in *C. persicum* by hybridization or using a spontaneous mutation in the species because vegetative reproduction and interspecific hybridization were difficult. Breeding programs using only crosses and spontaneous mutations have limited possibilities to alter phenotypes in the cyclamen, but these programs have successfully produced many cultivars, including F1 hybrids. Therefore, research on new breeding techniques is desirable and brings great possibilities to cyclamen breeding programs (Takamura, 2007).

#### Breeding purposes of cyclamen;

- Number of Flowers and Uniform Flowering,
- Number of Leaves and Plant Form,
- Shelf Life,
- Hot and Cold Tolerance,
- Resistance to Diseases and Pests,
- Earliness,
- Fertilizer Consumption,
- Seed Germination and Dormancy are among the breeding purposes (Curuk et al. 2021).

## 2.2. Breeding Methods

### 2.2.1. Classic Breeding

*Cyclamen persicum* in the genus *Cyclamen* is the only commercial species, it has evolved through intraspecific variation through cultivar crosses and natural mutation, resulting in the production of many cultivars (Legro, 1959). *Cyclamen* species other than *C. persicum* have not been used as commercial plants due to hybrid viability and hybrid sterility. Wild *Cyclamen* species have many desirable properties such as cold resistance, disease resistance and floral fragrance and are therefore potential gene sources for breeding *C. persicum* (Ishizaka, 2008). If we look at the studies on flower scent in *cyclamen* breeding; They performed an interspecies cross on *Cyclamen persicum* and *Cyclamen purpurascens* and used the technique of ovule culture *in vitro* to rescue abortive embryos (Ishizaka and Uematsu, 1995). Disease resistance breeding studies; They performed hybridization studies using *C. persicum* and *C. greacum* species, and the resulting abortive hybrid embryos were placed in ovule culture (Ishizaka, 1996). Interspecific hybrids of *C. persicum* cultivars and *C. hederifolium* have been produced (Ishizaka & Uematsu, 1992;1994). In cold resistance breeding studies; Tutuncu et al. (2020), performed reciprocal hybridization of *Cyclamen coum* and Commercial *Cyclamen persicum* genotypes. The immature embryos in the resulting immature seed pods were cultured *in vitro*. Due to mature or immature embryos formed after hybridization in *cyclamen*, there is no material loss in breeding using biotechnological methods and positive results can be obtained. Ishizaka & Uematsu (1992), As a result of their studies, they reported that embryo abortive after fertilization would be overcome with ovule culture and hybrid plants would be obtained.

### 2.2.2. Mutation Breeding

Nuclear radiation and chemical mutation are used to obtain new varieties and are an important method of breeding vegetatively propagated species, especially ornamental plants (Hvoslef-Eide and Munster, 2007). It is an important breeding method used to create new colors and shapes in ornamental plants, change plant shape, increase shelf life, provide resistance against insects and diseases, or create variations that will aid further breeding programs (Sheela and Sheena, 2014). As of 2021, there are 3387 mutant plant varieties registered in the FAO/IAEA (International Atomic Energy Agency) database (Anonim, 2022). Most of the varieties have been created by ionizing radiation, primarily using gamma rays (Tanaka et al. 2010). Mutation breeding studies in *Cyclamen* are given in Table 1.

**Table 1.** Mutation Breeding Studies in *Cyclamen*

Type or Variety	Material	Procedure	Reference(s)
<i>C. persicum</i> cv.	Callus, somatic embryo, plantlet; Tubers (8-15mm)	$^{12}C^{6+}$ ion radiation (10, 20, 40, 60 and 80 Gy) For the tubers; 4, 8, 12 and 16 Gy	Sugiyama et al. (2008)
<i>C. Persicum</i> cv.	Seeds germinated <i>in vitro</i>	320-MeV carbon ion beam (0, 0.5, 1, 2, 4, 8, 16 Gy)	Kondo et al. (2009)

### 2.2.3. Biotechnological Methods

In addition to frequently used propagation techniques such as tissue culture, micropropagation, somatic embryogenesis, organogenesis, techniques such as haploid plant production (ovule-ovarium, microspore-anther culture), embryo recovery and gene transformation are used to shorten

the duration of breeding studies and increase success. Through organogenesis and/or embryogenesis, almost any type of plant cell can be used, but optimal growing conditions must be found (Seguí-Simarro et al. 2021). The use of biotechnological methods in breeding brings short-term and permanent solutions to many problems that cannot be solved by classical methods (Kurtar and Balkaya, 2017). In addition to classical methods, biotechnological methods have recently been widely used to shorten the breeding period and develop new varieties in *Cyclamen* (Curuk et al. 2021).

The purpose of haploidization is to obtain homozygous pure lines in plants. Haploid plants with the chromosome number (n) in the gamete cells are obtained. For this purpose, haploid plants can be developed from male gametes (androgenesis), and haploid plants can be formed from female gametes (Ellialtıoglu et al. 2012). The haploidization technique includes ovule-ovarium, microspore-anther culture and irradiated pollen applications (Seguí-Simarro et al. 2021). In addition, its use in cyclamen breeding is quite limited. Studies done on cyclamen are given in Table.2. In addition, Sevindik (2018) and Al-Khafaj (2021) performed anther culture to obtain haploid plants in *cyclamen*.

**Table 2.** Biotechnological Studies in *Cyclamen* Breeding

Female	Male	Technique	Reference(s)
	<i>C. persicum</i>	Anther Culture	Pirrie (1985)
	<i>C. hederifolium</i>	Ovule Culture	Ishizaka and Uematsu (1992;1994)
<i>C. persicum</i>	<i>C. persicum</i>	Anther Culture	Ishizaka and Uematsu (1993)
	<i>C. purpurascens</i>	Ovule Culture	Ishizaka and Uematsu (1995a;1995b)

<i>C. greacum</i>	Ovule Culture	Ishizaka (1996)
<i>C. purpurascens</i>	Ovule Culture	Ewald (1996)
<i>C. purpurascens</i>	Ovule Culture	Ewald et al. (1998)
<i>C. purpurascens</i>	Anther Culture	Ishizaka (1998)
<i>C. coum</i>	Ovule Culture	Tutuncu et al. (2021)

## CONCLUSION

*Cyclamen*, which is in the Myrsinaceae family, is one of the most important geophyte plant groups and is a popular potted ornamental plant with economic importance in many countries. It is also used as a cut flower. The purpose of cyclamen breeding is to attract the attention of the market, which is to grow new, original and disease-resistant plants. Breeding criteria; Features such as flower color and size, plant size, shape, number of leaves, leaf type, stem length, stem thickness are used for the development of new varieties. There are different breeding methods for cyclamens. The first of these is the hybridization method, which is called classical breeding, and it is used more often than other breeding techniques in cyclamens. Classical breeding also complicates this method, as hybrid incompatibilities occur. The second is the mutation breeding method. It makes use of different rays and chemicals and has become popular lately as it is an effective way of producing new plant varieties. When we look at the mutation breeding studies in cyclamen, there is no registered mutant variety in the *cyclamen* genus. Recently, the importance of biotechnology has increased in breeding studies. Therefore, fields such as genetic engineering and gene transformation, that is, biotechnological methods, will make positive contributions to future breeding studies. In addition to these techniques, using the new generation sequencing

technology, which has been used in other plants, in cyclamen breeding will shorten the breeding period and ensure rapid success. Also, a large number of *Cyclamen* plants can be obtained by using a bioreactor *in vitro*, so mass propagation can be achieved with the developed protocols. In addition, there should be cooperation and exchange of information with researchers working on cyclamen around the world so that the studies are not repeated.

### **ACKNOWLEDGEMENT**

The authors thank Ghassan Zahid and Ibrahim Bayatli for their helpful suggestions in the preparation of this article; We are grateful to Pembe Evcı Curuk and Tansu Hasturk for the photos.

## REFERENCES

- Al-Khafaji, M. N. S. (2021). Production Of Microspore-Derived Plants By Anther Culture Of (*Cyclamen coum*), Van Yuzuncu Yil University Institute Of Natural And Applied Sciences, M.Sc. Thesis, 77.
- Altunkeyik, H., Gülcemal, D., Masullo, M., Alankus-Caliskan, O., Piacente, S., & Karayildirim, T. (2012). Triterpene saponins from *Cyclamen hederifolium*. *Phytochemistry*, 73, 127-133.
- Alp, H. A. (2020). Researches on somatic embryogenesis and synthetic seed production of tubers of some *cyclamen* species grown in Turkey. Cukurova University Institute of Science and Technology, Ph.D. Thesis, 218.
- Amini, L. (2014). The effects of anther and ovule culture methods on embryo stimulation in some *Cyclamen* species (*C. cilicium*, *C. persicum* ve *C. hederifolium*). Cukurova University, Graduate School of Natural and Applied Sciences, Adana, Master Thesis, 57.
- Anonim (2022). International Atomic Energy Authority, Mutant Variety Databases <https://mvd.iaea.org/>.
- Aydin, C., Cennet, Ozay., & Mammadov, R. (2014). Studies on *Cyclamen* L. Species Distributed in Turkey. Hacettepe University Journal of The Faculty Of Pharmacy, (2), 96-112.
- Balkaya, A., Izgi Sarac, Y., & Tutuncu, M. (2021). Breeding Ornamental plants (Classical and Biotechnological Methods). Gece Kitaplığı.
- Bian, F., Zheng, C., Qu, F., Gong, X., & You, C. (2010). Proteomic analysis of somatic embryogenesis in *Cyclamen persicum* Mill. *Plant Molecular Biology Reporter*, 28(1), 22-31.
- Calis, I., Yürüker, A., Tanker, N., Wright, A. D., & Sticher, O. (1997). Triterpene saponins from *Cyclamen coum* var. *coum*. *Planta medica*, 63(02), 166-170.
- Compton, J. A., Clennett, J. C. B., & Culham, A. (2004). Nomenclature in the dock. Overclassification leads to instability: a case study in the horticulturally important genus *Cyclamen* (Myrsinaceae). *Botanical Journal of the Linnean Society*, 146(3), 339-349.
- Cornea-Cipcigan, M., Pamfil, D., Sisea, C. R., & Mărgăoan, R. (2020). Gibberellic acid can improve seed germination and ornamental quality of selected *Cyclamen* species grown under short and long days. *Agronomy*, 10(4), 516.
- Culham, A. (2006). A phyloclimatic study of *Cyclamen*. *BMC Evolutionary Biology*, 6.
- Curuk, P., (2013). Morphological and Molecular Characterization of *Cyclamen* Species Growing Naturally in Adana and Its Surroundings. Cukurova University Institute of Science and Technology, Ph.D. Thesis 216.
- Curuk, P., Sogut, Z., Bozdogan, E., Izgu, T., Sevindik, B., Tagipur, E. M., Da Silva, J. A. T., Serçe, Kaçar Y. A., S., Mendi, Y. Y. (2015) Morphological characterization of *Cyclamen* sp. grown naturally in Turkey: Part I. *S Afr J Bot* 100, 7-15.
- Curuk, P., Sogut, Z., Izgu, T., Sevindik, B., Tagipur, E. M., da Silva, J. A. T., ... & Mendi, Y. Y. (2016). Morphological characterization of *Cyclamen* sp. grown naturally in Turkey: Part II. *Acta Sci. Pol. Hortorum Cultus*, 15(5), 205-224.

- Curuk, P., Salman, A. & Mendi, Y. Y. (2021). Ornamental Breeding Plants (Species). Gece Books.
- Dall'acqua, S., Castagliuolo, I., Brun, P., Ditadi, F., Palu, G. & Innocenti, G. (2010) Triterpene Glycosides with *in vitro* Anti-Inflammatory Activity from *Cyclamen repandum* tubers, Carbohydrate Research, 30, 345- 709.
- Debussche, M. A. X., Garnier, E., & Thompson, J. D. (2004). Exploring the causes of variation in phenology and morphology in Mediterranean geophytes: a genus-wide study of *Cyclamen*. Botanical Journal of the Linnean Society, 145(4), 469-484.
- Dillen, W., Dijkstra, I., & Oud, J. (1996). Shoot regeneration in long-term callus cultures derived from mature flowering plants of *Cyclamen persicum* Mill. Plant cell reports, 15(7), 545-548.
- Doorenbos, J. (1950). The history of the " Persian" *cyclamen* (No. 88).
- Ekim, T., Koyuncu, M., Guner, A., Erik, S., Yildiz, B., Vural, M. (1991). Taxonomic and Ecological Research on Geophytes of Turkey's Economic Value, T. C. Ministry of Agriculture, Forestry and Rural Affairs, General Directorate of Forestry, Department of Business and Marketing, Ankara.
- Elliältioglu, S., Basay, S., & Kusvuran, S. (2012). Investigation of the relationship between pollen dimorphism and anther culture in eggplant. Tarım Bilimleri Araştırma Dergisi, (1), 149-152.
- Erturk, O. (2022). On the Development of *Cetonia aurata* L. (Coleoptera, Scarabaeoidea, Cetoniidae) *Cyclamen coum* subsp. Antifeedant and Toxic Effects of Coum Miller Plant Root Extracts. Anadolu Tarım Bilimleri Dergisi, 37(2), 243-262.
- Ewald, A. (1996). Interspecific hybridization between *Cyclamen persicum* Mill, and *C. purpurascens* Mill. Plant Breeding, 115(3), 162-166.
- Ewald, A., Orlicz-Luthardt, A., Winkelmann, T., & Schwenkel, H. G. (1998). Interspecific hybrids of *Cyclamen persicum* Mill. X *Cyclamen purpurascens* Mill.: propagation, somaclonal variation, resistance to Fusarium wilt and suitability as an outdoor crop. In XIX International Symposium on Improvement of Ornamental Plants 508 (pp. 309-310).
- Grey-Wilson, C. (2015). *Cyclamen*. A Guide for Gardeners, Horticulturists and Botanists. Pavilion Books, Batsford.
- Gundogan, M.T. (2003). Some Phytochemical Studies on *Cyclamen mirabile* Hildebr. and *Cyclamen trochopteranthum* O. Schwarz Species. Muğla University, Institute of Science and Technology, Master Thesis.
- Hvoslef-Eide, A. K., & Munster, C. (2007). Begonia. In Flower Breeding and Genetics (pp. 241-275). Springer, Dordrecht.
- Horn, W. (2002). Breeding methods and breeding research. In Breeding for ornamentals: classical and molecular approaches (pp. 47-83). Springer, Dordrecht.
- Horn, W. (2004). The patterns of evolution and ornamental plant breeding. Acta Hort. 651, pp. 19–31 <https://doi.org/10.17660/ActaHortic.2004.651.1>.
- Izgu, T., Sevindik, B., Curuk, P., Simsek, O., Aka Kacar, Y., Teixeira da Silva, J. A., Mendi, Y. Y. (2016). Development of an efficient regeneration protocol for

- four *Cyclamen* species endemic to Turkey. *Plant Cell, Tissue and Organ Culture*, 127(1), 95-113.
- Ishizaka H, Uematsu J. (1992). "Production of interspecific hybrids of *Cyclamen persicum* Mill. and *C. hederifolium* Aiton. by ovule culture", *Japanese Journal of Breeding*, 42(2), 353-366.
- Ishizaka, H., Uematsu, J. 1993. Production of Plants from Pollen in *Cyclamen persicum* Mill. Through Anther Culture. *Japanese Journal of Breeding*, 43 (2), 207-218.
- Ishizaka H, Uematsu J. (1994) Amphidiploids between *Cyclamen persicum* Mill. and *C. hederifolium* Aiton induced through colchicine treatment of ovules *in vitro* and plants. *Breed Sci* 44: 161–166
- Ishizaka, H., Uematsu, J. (1995a). Interspecific Hybrids of *Cyclamen persicum* Mill. and *C. purpurascens* Mill. Produced by Ovule Culture. *Euphytica*, 82 (1), 31-37.
- Ishizaka, H., & Uematsu, J. (1995b). Amphidiploids between *Cyclamen persicum* Mill. and *C. purpurascens* Mill. induced by treating ovules with colchicine *in vitro* and sesquidiploids between the amphidiploid and the parental species induced by conventional crosses. *Euphytica*, 86(3), 211-218.
- Ishizaka, H. 1996. Interspecific Hybrids of *Cyclamen persicum* and *C. graecum*. *Euphytica*, 91, 109-117.
- Ishizaka, H., 1998. Production of Microspore-Derived Plants by Anther Culture of an Interspecific F1 Hybrid Between *Cyclamen persicum* and *C. purpurascens*. *Plant cell, Tissue and Organ Culture*, 54 (1), 21-28.
- Jalali, N., Naderi, R., Shahi-Gharahlar, A., & da Silva, J. A. T. (2012). Tissue culture of *Cyclamen* spp. *Scientia horticulturae*, 137, 11-19.
- Kayıkçı, S., Altay, V., & Güzel, Y. (2012). A Study on Some Geophytic Plant Species Distributing in Hatay Province. *Biyoloji Bilimleri Araştırma Dergisi*, 5(2), 139-143.
- Kocak, M., Izgu, T., Sevindik, B., Tutuncu, M., Curuk, P., Simsek, O., ... & Mendi, Y. Y. (2014). Somatic embryogenesis of Turkish *Cyclamen persicum* Mill. *Scientia Horticulturae*, 172, 26-33.
- Kocak, M. (2019). Investigation of regeneration by somatic embryogenesis method in *Cyclamen coum*. Cukurova University Institute of Science and Technology, Ph.D. Thesis, 123.
- Kondo, E., Nakayama, M., Kameari, N., Tanikawa, N., Morita, Y., Akita, Y., ... & Ishizaka, H. (2009). Red-purple flower due to delphinidin 3, 5-diglucoside, a novel pigment for *Cyclamen* spp., generated by ion-beam irradiation. *Plant biotechnology*, 26(5), 565-569.
- Kurtar, E. S., & Balkaya, A. (2017). The Using of Biotechnology in Cultivar Breeding of *Cucurbita* Vegetable Species. *Bahçe*, 46(2), 39-49.
- Legro, R. A. H. (1959). The cytological background of cyclamen breeding. Wageningen University and Research.
- Mammadov, R. (2014). Secondary metabolites in seed plants. Nobel Pres, Ankara-Turkey., pp.93-115.
- Mayer, L. (1956). Growth and organ formation of *in vitro* cultivated segments of *Pelargonium zonale* and *Cyclamen persicum*. *Planta*, 47(4. H), 401-446.

- Mazouz, W. & Djeddi, S. (2013). A biological overview on the Genus *Cyclamen*. *European Journal of Scientific Research*, 110(1), 7-22.
- Metin, H. (2012). The characterization of active components of *Cyclamen graecum* link. Extracts and the determination of their antioxidant and histological effects. Pamukkale University Graduate School of Natural and Applied Sciences, Master Thesis, 82.
- Mohammed, G. J., Hameed, I. H., & Kamal, S. A. (2018). Anti-inflammatory effects and other uses of *Cyclamen* species: A review. *Indian Journal of Public Health Research and Development*, 9(3), 206-211.
- Motoyasu O, Takiko S (1991) Somatic embryogenesis and plant regeneration from *Cyclamen persicum* Mill. leaf cultures. *Plant Tissue Culture Letters* 8:121–123.
- Murasaki, K., & Tsurushima, H. (1987, August). Improvement on clonal propagation of *cyclamen in vitro* by the use of etiolated petioles. In International Symposium on Propagation of Ornamental Plants 226 (pp. 721-724).
- Neumaier, D., Bausbach, E., Simon, M., Tisch, M., & Winkelmann, T. (2009). Effect of time of harvest, water quality and cut flower food on vase life of *Cyclamen* cut flowers. *Acta Horticulturae*, 847: 269-274.
- Pieroni, A., Howard, P., Volpato, G., & Santoro, R. F. (2004). Natural remedies and nutraceuticals used in ethnoveterinary practices in inland southern Italy. *Veterinary research communications*, 28(1), 55-80.
- Pirrie, A., (1985). The Use of Haploid Systems in Plant Genetic Manipulation (Doctoral dissertation, University of Nottingham).
- Prange, A. N. S., Bartsch, M., Serek, M., & Winkelmann, T. (2010). Regeneration of different *Cyclamen* species via somatic embryogenesis from callus, suspension cultures and protoplasts. *Scientia horticulturae*, 125(3), 442-450.
- Read, P. E., & Preece, J. E. (2007, September). Micropropagation of ornamentals: The wave of the future?. In III International Symposium on Acclimatization and Establishment of Micropropagated Plants 812 (pp. 51-62).
- Schwenkel, H. G., & Grunewaldt, J. (1987, August). In vitro propagation of *Cyclamen persicum* Mill. In International Symposium on Propagation of Ornamental Plants 226 (pp. 659-662).
- Seguí-Simarro, J. M., Jacquier, N., & Widiez, T. (2021). Overview of *in vitro* and *in vivo* doubled haploid technologies. *Doubled haploid technology*, 3-22.
- Sevindik, B. (2018). Obtaining haploid plant via anther and ovule culture techniques in *Cyclamen*. Cukurova University Institute of Science and Technology, Ph.D. Thesis, 175.
- Seyring, M., Ewald, A., Mueller, A., & Haensch, K. T. (2009). Screening for propagation suitability *in vitro* of different *Cyclamen* species. *Electronic Journal of Biotechnology*, 12(4), 10-11. doi:10.4067/S0717-34582009000400010
- Shafiei-Masouleh, S. S. (2022). Use of magnetic nano-chitosan as bio-fertilizer to reduce production period in three *cyclamen* cultivars. *Journal of Soil Science and Plant Nutrition*, 22(1), 281-293.
- Sheela, V. L., Sheena, A., Tomlekova, N. B., Kozgar, M., & Wani, M. R. (2014). Novel trends and achievements in breeding of tropical ornamental crops

- especially orchids and anthuriums: the mutation breeding approach. Mutagenesis: exploring genetic diversity of crops. Wageningen, Netherlands, Wageningen Academic Publishers, 141-158.
- Speroni, E., Cervellati, R., Costa, S., Dall'Acqua, S., Guerra, M. C., Panizzolo, C., ... & Innocenti, G. (2007). Analgesic and antiinflammatory activity of *Cyclamen repandum* S. et S. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 21(7), 684-689.
- Sugiyama, M., Saito, H., Ichida, H., Hayashi, Y., Ryuto, H., Fukunishi, N., ... & Abe, T. (2008). Biological effects of heavy-ion beam irradiation on cyclamen. *Plant biotechnology*, 25(1), 101-104.
- Tagipur, M. E., Seker, G., Teixeira da Silva, J. A., & Yalcin Mendi, Y. (2016). Somatic embryogenesis, cryopreservation, and *in vitro* mutagenesis in Cyclamen. In *Somatic embryogenesis in ornamentals and its applications* (pp. 155-167). Springer, New Delhi.
- Takamura, T. & Miyajima, I. (1997). Micropropagation of *Cyclamen persicum* Mill. In: Y.P.S. Bajaj (Ed.), *Biotechnology in Agriculture and Forestry*, Vol. 40, High-tech and Micropropagation IV. Springer, Berlin Heidelberg, pp. 96-112.
- Takamura, T. (2007). *Cyclamen*. In *Flower breeding and genetics*(pp. 459-478). Springer, Dordrecht.
- Tanaka, A., Shikazono, N., & Hase, Y. (2010). Studies on biological effects of ion beams on lethality, molecular nature of mutation, mutation rate, and spectrum of mutation phenotype for mutation breeding in higher plants. *Journal of radiation research*, 51(3), 223-233.
- Tanker, N., Turkoz, S. (1984). *Cyclamen cilicicum* Boiss. Et Heldr var. *intaminatum* Meikle Üzerinde Morfolojik ve Anatomik Araştırmalar, Gazi Ecz. Fak. Der., 1(2):79.
- Tutuncu, M. , Izgu, T. , Sevindik, B. & Mendi, Y. (2020). Investigation of Interspecies Hybridization Possibilities in *Cyclamen*. *Çukurova Tarım ve Gıda Bilimleri Dergisi*, 35 (2), 133-142.DOI: 10.36846/CJAFS.2020.27
- Van Huylenbroeck, J. (2020). Breeding for sustainable ornamental plants. *Acta Horticulturae* 1288 (pp. 1-8). <https://doi.org/10.17660/ActaHortic.2020.1288.1>
- Yesson, C., & Culham, A. (2006). A phyloclimatic study of *Cyclamen*. *BMC Evolutionary Biology*, 6(1), 1-23.
- Yesson, C., & Culham, A. (2011). Biogeography of *Cyclamen*: an application of phyloclimatic modeling. *Climate change, ecology and systematics*, 265-279.
- Yalcın-Mendi Y, Curuk P. (2016). *Türkiye Florası Siklamenleri*. Adana. ISBN-13: 978-6050307160.
- Yaldız, G., Yuksek, T., & Sekeroglu, N. (2010). Medicinal and Aromatic Plants in the Flora of Rize Province and Their Usage Areas. III. National Black Sea Forestry Congress, 3, 1100-1114.
- Zeybek, N. and Zeybek, U. (1994). *Pharmaceutical Botany*, Ege Univ. Ecz. Fak. Yay. No:2, Bornova-Izmir.

- Wicart, G., Mouras, A., & Lutz, A. (1984). Histological study of organogenesis and embryogenesis in *Cyclamen persicum* Mill. tissue cultures: Evidence for a single organogenetic pattern. *Protoplasma*, 119(3), 159-167.
- Winkelmann, T. (2010). Clonal propagation of *Cyclamen persicum* via somatic embryogenesis. In *Protocols for in vitro propagation of ornamental plants* (pp. 281-290). Humana Press.
- Winkelmann T, Meyer L, Serek M (2003) Maturation and desiccation of somatic embryos of *Cyclamen persicum*. *Acta Horticulturae* 612, 27-34.
- Winkelmann, T., & Serek, M. (2005). Genotypic differences in callus formation and regeneration of somatic embryos in *Cyclamen persicum* Mill. *Euphytica*, 144(1), 109-117.
- Winkelmann, T., Ilczuk, A., & Wartenberg, S. (2010). Micropropagation through somatic embryogenesis of *Cyclamen persicum* Mill. genotypes for cut flower production—feasibility study. *Prop. Ornam. Plants*, 10, 237-245.
- Wellensiek SJ (1959). The effect of inbreeding in cyclamen, *Euphytica* 8, 125-130.

## CHAPTER 12

# RESPONSES OF PLANTS UNDER ABIOTIC STRESS AT THE *IN VITRO* AND MOLECULAR LEVEL IN SUSTAINABLE AGRICULTURE

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## INTRODUCTION

Sustainable agriculture has three basic components that have global effects: social, economic, and environmental factors. Ensuring sustainability is possible by maintaining the balance among these components (Eryılmaz, Kılıç; 2018). Among its aims are to reduce environmental damage while maintaining productivity in agriculture, to contribute to the economy, and to develop practices for this purpose (Turhan, Şule; 2005). Sustainable agriculture for developing countries; while it is an important element in terms of food safety, it can be determined as eliminating the negative effects of environmental quality and the resources used on the health of living beings in developed countries. Conservation of natural resources is very important in meeting people's food needs.

With the increase in the population of the world, the demand for food and raw materials increases day by day. Different stress factors also contribute to the reduction in plant production. High yields are obtained in agricultural systems where chemicals such as pesticides, herbicides, fossil fuels, and fertilizers are used with high inputs. However, the use of these inputs impairs the ecology, the environment, and the soil health, while polluting groundwater. For this reason, it is very important to use or breed varieties that are productive with less input, resistant to biotic and abiotic environmental conditions, or tolerant for the permanence of sustainability (Bharadwaj, 2016).

Plants are significantly influenced by various abiotic stress factors including salinity, drought, high temperature and chemical pollution. However, duration and intensity of such stressors designate the stress-induced metabolic, physiological, morphological and molecular changes in the plant. Plant response to abiotic stressors is widely assessed through *in vitro* selection. It is a common biotechnological method employed in plant breeding programs. The *in vitro* selection could be applied at different plant growth

stages including protoplast, cell suspension, callus formation, root and shoot regeneration. The stage in which *in vitro* selection is to be applied is largely designated by selective agent to be used. Unlike research in field or greenhouse conditions, *in vitro* techniques focus on the investigation of the factor and elimination of the effects of other factors in the environment (Hadrami et al., 2011).

In the evolutionary process, different mechanisms are developed by the plants to adapt and survive under various biotic and abiotic stressors. Plants regulate certain gene sets against these stresses. They induce signaling pathways activating kinase ladders, ion channels, production and accumulation of reactive oxygen types and hormones after sensing a stressor (Pérez-Clemente and Gómez-Cadenas, 2012).

To improve plant stress tolerance, resistance genes have been incorporated into the plants through conventional breeding methods. However, such methods are not sufficient in some cases to provide desired improvements in plant resistance or tolerance to various stressors (Maleki et al., 2019).

On the other hand, *in vitro* tissue culture techniques allowed researchers to develop new tools to address important issues about plant growth and development, stress resistance or tolerance. Such issues are the key parameters of sustainable agriculture. *In vitro* stress studies yielded significant outcomes for detecting plant response to various stressors and elucidating different resistance or tolerance mechanisms (Zekai et al., 2022). The *in vitro* selection process with different selective agents offered significant tools for plant breeders to better understand plant stress response and to develop stress-resistant or tolerant plants able to perform well under different stress conditions.

Abiotic stressors have complex and quite variable natures; thus, it is highly challenging to elucidate plant response to abiotic stressors directly in the field or under greenhouse conditions. Therefore, *in vitro* tissue culture techniques are often employed to analyze plant physiology and biochemistry under abiotic and biotic stress conditions in a short time (Pérez-Clemente and Gómez-Cadenas, 2012). For abiotic stress screening and management in crops, nanomaterials are also applied. Such nanomaterials offer low-cost tools with little energy requirement.

Abiotic stressors significantly restrict plant growth and development, thus yield and quality accordingly. They also result in serious losses in crop yield and quality. Various nanoparticles are employed to protect plants against abiotic stressors and in this way, to improve plant performance under stress conditions. For sustainable agriculture, nanotechnology is an important tool to recover problems about abiotic stress (Khan and Upadhyaya, 2019). Nanotechnology and *in vitro* abiotic stress studies have also started to take an important place in this regard.

This chapter provides information about the potential use of *in vitro* culture techniques for plant response to abiotic stressors and highlights the advantages of such techniques over conventional methods.

### **1. *In vitro* Applications of Abiotic Stress**

Thanks to plant tissue culture techniques, agricultural and ornamental plants are produced and take an active role in the manipulation of plants for improved agronomic performance. *In vitro* tissue culture has attracted great interest in recent years, as they increase genetic diversity and help the cultivation of advanced varieties, as well as allows physiological and genetic processes to be studied (Wani et al., 2010).

*In vitro* abiotic stress is the application of abiotic stressors (drought, low and high temperature, cold, salinity, iron chlorosis) *in vitro* (Figure 1).

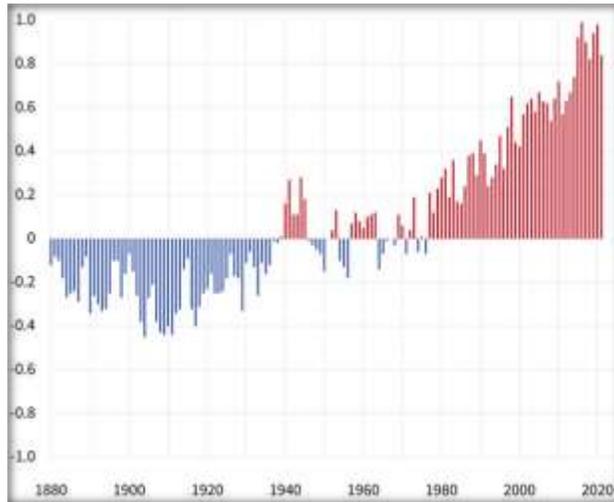


**Figure 1.** *In Vitro* Abiotic Stress Factors

In field and greenhouse conditions, many abiotic stress trials conducted from the past to the present have been researched on different plants and have contributed greatly to breeding studies and continue to do so. Investigation of abiotic stress-induced changes in plants with *in vitro* techniques offers many advantages. The most important of these advantages is that the controlled environment is easier and in a smaller area. Another advantage is that observations can be made more easily. On the other hand, the need for more costly and trained personnel compared to field and greenhouse trials, and the possibility of contamination is among its disadvantages.

### **1.1. *In vitro* Drought Stress**

Drought stress significantly influence crop production, yield and quality. The situation becomes more serious with the increasing population and global climate change (Figure 2). Due to decreasing water levels, water deficit is becoming more frequent in irrigated areas (Wani et al., 2010).



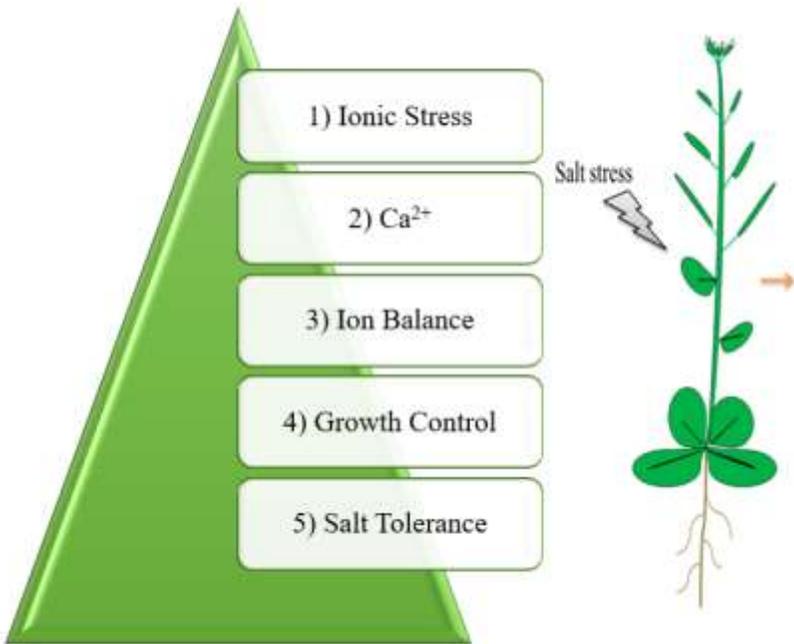
**Figure 2.** Temperature Change in the World by Years (Each number in the left column represents Celsius changes) (<https://www.climate.gov>.)

In the plants under drought stress, growth and development, yield, membrane integrity, pigment contents, osmotic compatibility, water relations, and photosynthetic activities are affected. Plants under drought stress accumulate soluble substances such as proline, sucrose, soluble carbohydrates, and glycine betaine to maintain cell turgor. Osmotic balance is provided by these solutes (Hajihashemi and Ehsanpour, 2013).

Adverse impacts of drought stress and the increasing severity of drought around the world have contributed to the acceleration of *in vitro* studies in this direction. Polyethylene glycol (PEG) with different concentrations and different molecular weights is commonly used to simulate drought stress *in vitro*. It was reported that PEG induced drought stress and didn't have any toxic impacts (Meher et al., 2018). In addition to PEG, mannitol solution is also used for *in vitro* drought stress generation. Mannitol is polyhydric alcohol that causes drought stress (Soetaert et al., 1999).

## 1.2. *In vitro* Salinity Stress

Salinity stress is generally generated through excessive concentrations of  $\text{Na}^+$  and/or  $\text{Cl}^-$  ions. It strictly restricts crop production, plant growth and development (Parihar et al., 2015) (Figure 3).



**Figure 3.** Salinity Stress and Plant Response to Stress

Dissolved salts in soil solution can inhibit plant growth and development through two different mechanisms. High salt concentrations hinder plant water uptake, thus reduces plant growth, so called as water-deficient effect. Excessive salt concentration through transpiration results in cell damage and growth recession, so called as ion-excess effect (Parihar et al., 2015; Greenway and Munns, 1980). To investigate the impacts of salt stress *in vitro*, different concentrations of  $\text{NaCl}$  are added to growing medium.

### **1.3. *In vitro* Heat Stress**

Temperatures above optimum values create stress on plants as in all living organisms and are so called as heat stress (Kotak et al., 2007). Heat stress has various negative effects on plant growth and development, physiological processes, crop yield and quality (Hasaruzzaman et al., 2013). Heat stress disrupts cellular homeostasis, thus causes serious regression in growth and development in plants and even leads to their death of them. It causes widespread agricultural losses worldwide (Kotak et al., 2007).

Besides *in vivo* research, abiotic stress (heat stress) trials can also be conducted *in vitro* and plant response at different temperatures and times can be examined. Thus, plants can be classified as hardy, tolerant and sensitive. *In vitro* temperature experiments, in the climatization chambers of the plants transferred to the nutrient medium, are carried out by incubating them at high temperatures instead of suitable growing temperatures.

#### **1.3.1 *In vitro* Cold Stress**

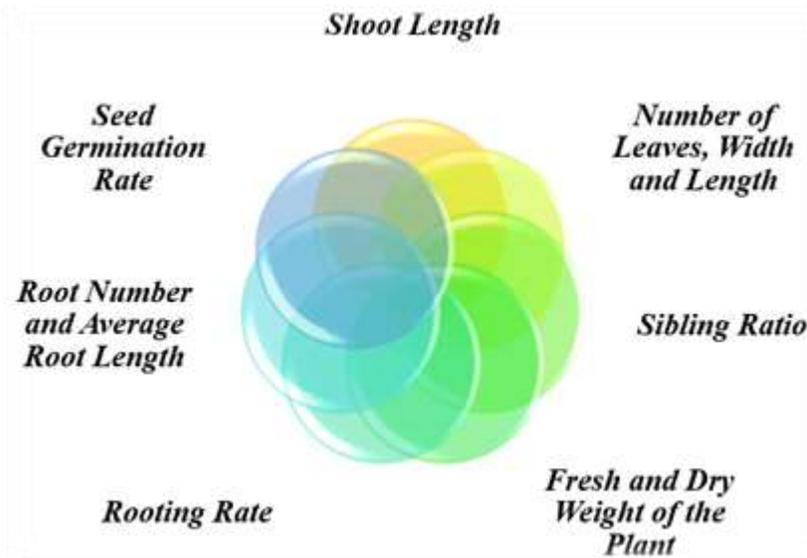
Cold stress, an abiotic stressor, results in serious reductions in crop yield and has adverse effects on plant physiology and biochemistry. Plant survival and distribution are affected by low temperatures, which include cooling (0–10 °C) and freezing (<4 °C) (Megha et al., 2018). Plants are poikilothermic organisms. In other words, the temperature of plant tissues varies depending on the ambient temperature. Plants, which cannot move sense the changes in the temperature of the environment they live in and adjust their metabolic reactions according to the new temperature regime. Considering the evaluation of FAO in terms of environmental factors limiting agricultural activities, it is reported that most of the soils in our country are at risk of low-temperature stress (Doğru, 2019). In this case, thus, it is extremely important to research plants that are resistant to low temperatures. Low-

temperature studies can be carried out with plants induced at low temperatures instead of normal growing conditions with *in vitro* studies.

## **2. Changes in Plants After *In Vitro* Abiotic Stress**

When plants are in an environment that will cause them stress, it is first sensed through receptors on cell walls and membranes. After the perception of stress, various signals occur in their bodies. Signals are transferred by various pathways, causing alterations in gene expressions; because of these changes, various answers occur (Çetinkaya and Seçkin Dinler, 2021). When evaluated in terms of anatomy, plants show many changes in terms of organs and adverse environmental conditions. It changes its anatomical features to adapt to adverse environmental conditions with the main organ, especially roots, xylem, and leaves. Regardless of plant type and stress factor, plants show similar anatomical differences (Patakas, 2012). Depending on different growth and morphological stages, and the stresses in plants; it causes shorter shoot length, decreases and shrinkage of the number of leaves, changes in root number and length, decrease in plant fresh and dry weight, and decrease in flowering. As a result, it hurts yield. In addition, it affects the germination of seeds with the cessation of meristematic cell activity in young seedlings (Zulfiqar et al, 2019). Many of these parameters can be evaluated in a short time under different stress tests with *in vitro* studies.

Germination rates of seeds obtained from the fruits of plants grown under stress under *in vivo* conditions can be evaluated *in vitro*. In addition, when plants under stress are evaluated physiologically, it causes deterioration of the photosynthesis mechanism and a decrease in photosynthesis, and the stomata are closed (Farooq et al., 2009).



**Figure 5.** Examined Morphological Parameters of Plants Under Abiotic Stress *In vitro*

*In vitro* abiotic stress studies in many economically important agricultural species and ornamental plants have been conducting on for many years. Some of these studies are presented in Table 1.

**Table 1.** *In vitro* abiotic stress studies in some economically important agricultural species and ornamental plants

Species	Abiotic Stress Mechanism	Reference
Carrot ( <i>Daucus carota</i> L.)	Cadmium	Di Toppi et al., 1998
Cultivated and wild tomato species	Salt	Cano et al, 1998
Pear rootstocks	Drought and salt	Rahman et al., 2007
Peach rootstock	Fe deficiency	Lombardi et al., 2007
Tomato ( <i>Lycopersicon esculentum</i> Mill.)	Salt	Amini et al., 2007
Olive cultivars ( <i>Olea europaea</i> L.)	Salt	Bracci et al., 2008
Citrus species	Salt	Montoliu et al., 2009
Olive cultivars ( <i>Olea europaea</i> L.)	Drought	Faraloni et al., 2011
Apple rootstock	Salt	Bahmani et al., 2012
Potato ( <i>Solanum tuberosum</i> L.)	Fe deficiency	Legay et al., 2012

Grapevine ( <i>Vitis vinifera</i> )	Drought	Mehri et al., 2015
Tomato and related wild species	Salt	Zaki and Yokoi, 2016
Grapevine ( <i>Vitis vinifera</i> )	Drought	Cui et al., 2017
Citrus rootstocks	Drought	Şimşek, 2018
European Bluestar ( <i>Amsonia orientalis</i> Decne.)	Drought	Acemi et al., 2018
Citrus rootstocks	Drought	Şimşek et al., 2018
American grapevine rootstocks	Drought	Meşe and Tangolar, 2019
Banana ( <i>Musa acuminata</i> )	Simulated water deficit or salinity stress	Mahmoud et al., 2020
Apricot ( <i>Prunus armeniaca</i> )	Drought	Girici, 2021
Aubergine ( <i>Solanum melongena</i> )	Salt	Hannachi et al., 2021
Banana ( <i>Musa L.</i> )	Drought	Zekai et al., 2022
Pear rootstocks	Fe deficiency	Şahin et al., 2022
<i>Thymus lotocephalus</i>	Drought	Mansinhos et al., 2022

### 3. Changes in Molecular Characteristics Observed as a Result of Abiotic Stress Effects

Plant reactions to abiotic stress are complicated since stress sensed in one component may be responded by an alternative component (Tester and Davenport 2003). As a response to stress exposure, plants often evolve an abstinence treatment together with tolerance. Deep-rooted plants develop evasion mechanisms against drought, alters gene expressions and adjust metabolic pathways to improve plant tolerance and boost metabolic functions under stress conditions (Patakas 2012).

Transgenic approaches largely rely on biotechnological methodologies and offer important instruments in breeding programs. Recently, several stress adaptation mechanisms and gene families have been discovered. Such gene families could be manipulated in different combinations, ectopically expressed and transferred to different species. Therefore, the possibility of

transforming resistance or tolerance genes into major crops offers significant tools to plant breeders (Maleki 2019).

Plant response to osmotic action of salt largely resulted from cellular and metabolic processes developed against salinity stress. Osmo-protectant or osmo-regulation genes have successfully been used in development of drought-resistant plants. In some cases, glycine betaine intermediates are also transferred into plants to enhance drought and salinity resistance or tolerance of transgenic plants (Aslantaş 2010).

Manipulation of transcription factors (TFs), late embryogenesis abundant (LEA) proteins and antioxidant proteins are also used as different approaches that have been successfully developed in different crops to obtain plants resistant to abiotic stress through transgenesis. Besides these approaches, molecular markers are largely employed in genomic analysis for stress tolerance of plants. Resistance-related markers facilitate breeding programs conducted to improve plant tolerance to various stressors. Such an approach is especially powerful when polymorphic characters were targeted, as in most abiotic stress situations (Thudi 2021).

WRKY transcription factors are one of the largest transcription factor families in plants. It plays a very important role in plant growth and development, defense regulation and stress response. MYB proteins are major transcription factors that play significant roles in plant defenses against various stresses (Wu et al., 2019).

Also, some studies have obviously indicated that many transcription factors, concerning MYB, AP2/ERF, HD-Zip, WRKY, and NAC families, have been defined, described, and shown to attend in the regulation of plants' responses to abiotic stresses (Mizoi et al., 2012; Shao et al., 2015; Perotti et al., 2017; Erpen et al., 2018). Genetically modified plants overexpressing

these groups of genes have proposed cultivated resistance to different stresses (Erpen et al., 2018).

Plants are exposed to abiotic stresses such as drought and salinity and biotic stresses such as pathogens and fungi. TEs play critical roles in initiating genetic plasticity during plant-abiotic/biotic stress interactions. Many displaceable elements are activated in response to stress conditions, altering gene expression. Even though TEs do not prevent stress, they can change gene expression in response to different environmental stresses and regulate gene regulation under stress conditions (Sezer, 2018).

Although marker-assisted selection offers a reliable means of plant development and improvement, the potential use of marker-assisted selection to improve the stress tolerance of plants is highly restricted because few stress tolerance QTLs have been identified (Zaki 2016).

Genetic modification of higher plants through DNA promotion or transformation is a complicated issue. Transformation trials largely rely on cell and tissue culture at some point. Improvement of transformation techniques, and preventing tissue culture, have already been achieved in *Arabidopsis* and extended to several plants, plant regeneration from isolated cells or tissues *in vitro* is still in progress for different crops. Not only every transformation method is not suitable for all plants, but also plant species cannot be regenerated through all methods. Therefore, both an appropriate plant tissue culture and an adaptive plant transformation method must be found. Many agriculturally important species are currently being studied. Table 2 provides a list of species susceptible to *Agrobacterium*-mediated transformation (Thudi 2021).

**Table 2.** Genetic Transformation for Increased Resistance to Abiotic Stresses

Transgenic Plant Species	Stress	Transferred Genes	Reference
<i>C. microcarpa</i> Bunge, <i>Poncirus trifoliata</i> (L.) Raf., <i>Carrizo citrange</i> <i>C. sinensis</i> Osb. cv Hong Jiang	Cold	<i>CS-ACS1</i>	Wong et al, 2001
<i>Carrizo citrange</i>	Drought	<i>p5cs</i>	Molinari et al, 2004
<i>Poncirus trifoliata</i> L. Raf.	Salt	<i>AhBADH</i>	Fu et al, 2011
<i>Solanum lycopersicum</i> cv. PED	Salt	<i>TaNHX2</i>	Yarra et al, 2012
<i>Arabidopsis</i>	Salt	<i>GsMYB15</i>	Shen et al, 2018
<i>Arabidopsis</i>	Drought and Heat	<i>ZmWRKY106</i>	Wang et al, 2018
<i>Oryza sativa</i>	Drought	<i>ScMYBAS1</i>	Peixoto-Junior et al, 2018
<i>Arabidopsis</i>	Salt	<i>NAC57</i>	Yao et al, 2018
<i>Triticum aestivum</i>	Salt	<i>HvBADH1</i>	Li et al, 2019
<i>Oryza sativa</i>	Salt/Drought	<i>OsMYB6</i>	Tang et al, 2019
<i>Arabidopsis</i>	Drought and Salt	<i>ZmMYB3R</i>	Wu et al, 2019
<i>Gossypium hirsutum</i> L.	Drought and Salt	<i>GhGA2ox1</i>	Shi et al, 2019
<i>Arabidopsis</i>	Salt	<i>JcHDZ07</i>	Tang et al, 2019
<i>Arabidopsis thaliana</i>	Salt	<i>CrCOMT</i>	Zhang et al, 2019
<i>Medicago sativa</i> L.	Metal	ATP sulfurylase	Kumar et al, 2019
<i>Solanum lycopersicum</i> L.	Cold and Drought	<i>SiDHN</i>	Guo et al, 2019
<i>Arabidopsis</i>	Metal	<i>PpMT2</i>	Liu et al, 2020
<i>Arabidopsis</i>	Drought, Salt and Heat	<i>AVP1, RCA</i> and <i>AVP1/RCA</i>	Wijewardene et al, 2020
<i>Arabidopsis</i> and <i>Oryza</i> <i>sativa</i>	Drought	<i>PeTCP10</i>	Liu et al, 2020
<i>Arabidopsis</i> and <i>Glycine</i> <i>max</i>	Drought and Salt	<i>GmbZIP2</i>	Yang et al, 2020
<i>Lycopersicon esculentum</i> Mill	Cold	<i>LeCOR413PM2</i>	Zhang et al, 2021

### **3. Risk to Sustainable Agriculture by Abiotic Stress**

Abiotic stresses affected agricultural production. Worldwide, about 91% of agricultural lands are under stress and such stress factors result in about 50% loss in agricultural production. On-going climate change and global warming may facilitate the severity and adverse effects of abiotic stresses. The progress achieved in agronomical management and promotion of stress-resistant genotypes could alleviate such adverse effects of stressors on plants. Abiotic stress easily influences plant biochemical and physiological processes. Plant light utilization efficiency and photosynthetic activity should be increased to improve plant resistance against abiotic stress factors. Also, many antioxidant activations occur and enzymes may develop stress-based metabolites, preventing cellular damage. However, there is still a need for the development of basic adaptation strategies to increase plant stress tolerance levels.

Abiotic stress factors vary in constituents and each one result in serious damage on soil and environment, yield and quality losses. Today, about 90 percent of agricultural lands qualify as land with one or more stressors, while only 10 percent constitutes a non-stress zone. Plants may inherently develop biochemical, molecular, physiological and phenotypic adaptation mechanisms against abiotic stress. However, additional efforts are still needed to improve stress tolerance by promoting resource conservation technologies, genetically enhancing plant defenses, and adopting other approaches.

### **4. Conclusion**

New genetic sources are needed to improve salinity tolerance of the plants or more effective methods should be used to identify salt-tolerant genotypes. Despite the availability of efficient molecular tools for

manipulation of genetic sources, further effort is needed to transfer tolerance genes into existing varieties.

Drought stress impairs cell division and elongation seriously, thus hinders plant growth and development. Drought stress also reduces growth parameters, including plant height, leaf size and area, number of leaves and leaf area index (LAI) and has negative effects on physiological and morphological parameters. Breeding programs have achieved significant progress in identification and selection of drought-resistant genotypes.

Breeders are in search of fast, reliable, cost-effective and safe methods to grow plants that are particularly tolerant to abiotic stresses, due to the increasing demand for food worldwide. Also, the development of transgenic crops that are resistant to abiotic stresses are indispensable to ensure food security worldwide. For the promotion of sustainable agriculture in this section, *in vitro* selection may offer a suitable alternative to the breeders due to the afore-mentioned advantages.

As a result, micropropagation and *in vitro* selection are considered to be reliable solutions for several technical issues encountered throughout the growing periods of plants.

*In vitro* abiotic stress studies can be used not only to determine the resistance or tolerance of existing cultivars, but also to examine recently developed transgenic plants. However, by providing gene transfer to agricultural products with high commercial value and applying stress to these plants under *in vitro* conditions, their responses to stress at the morphological, biochemical and molecular level can be observed in a short time. The resulting data can be integrated into sustainable agriculture.

## REFERENCES

- Acemi, A., Duman, Y. A., Karakus, Y. Y., & Özen, F. (2018). Developmental and biochemical analyses of *in vitro* drought stress response in ornamental European Bluestar (*Amsonia orientalis* Decne.). *Folia Horticulturae*, 30(2), 405-414.
- Amini, F., Ehsanpour, A. A., Hoang, Q. T., & Shin, J. S. (2007). Protein pattern changes in tomato under *in vitro* salt stress. *Russian Journal of Plant Physiology*, 54(4), 464-471.
- Anjum, S. A., Ashraf, U., Zohaib, A., Tanveer, M., Naeem, M., Ali, I., Tabassum, T., & Nazir, U. (2017). Žemės ūkio augalų reakcija į sausros sukurtą stresą: Apžvalga. *Zemdirbyste*, 104(3), 267–276. <https://doi.org/10.13080/z-a.2017.104.034>
- Aslantaş, R., Karakurt, H., & Karakurt, Y. (2010). The Cellular and Molecular Mechanisms on Resistance to Low Temperatures in Plants. *Journal of Agricultural Faculty of Atatürk University*, 41(2), 157–167 in Turkish.
- Aydın Eryılmaz, G. and Kılıç, O. (2018). Sustainable Agriculture and Good Agricultural Practices in Turkey. *KSU J. Agric. Nat*, 21(4), 624–631 in Turkish.
- Bahmani, R., Gholami, M., Mozafari, A. A., & Alivaisi, R. (2012). Effects of salinity on *in vitro* shoot proliferation and rooting of apple rootstock MM. 106. *World Applied Sciences Journal*, 17(3), 292-295.
- Bracci, T., Minnocci, A., & Sebastiani, L. (2008). *In vitro* olive (*Olea europaea* L.) cvs Frantoio and Moraiolo microshoot tolerance to NaCl. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 142(3), 563-571.
- Cano, E. A., Pérez-Alfocea, F., Moreno, V., Caro, M., & Bolarín, M. C. (1998). Evaluation of salt tolerance in cultivated and wild tomato species through *in vitro* shoot apex culture. *Plant Cell, Tissue and Organ Culture*, 53(1), 19-26.
- Çetinkaya, H. and Seçkin Dinler, B. (2021). Stress Induced Defence Responses in Cell Wall Mechanisms in Plants. *Sinop Uni J Nat Sci* 6(2): 174-188 in Turkish.
- Cui, Z. H., Bi, W. L., Hao, X. Y., Li, P. M., Duan, Y., Walker, M. A., ... & Wang, Q. C. (2017). Drought stress enhances up-regulation of anthocyanin biosynthesis in grapevine leafroll-associated virus 3-infected *in vitro* grapevine (*Vitis vinifera*) leaves. *Plant disease*, 101(9), 1606-1615.
- di Toppi, L. S., Lambardi, M., Pazzagli, L., Cappugi, G., Durante, M., & Gabbrielli, R. (1998). Response to cadmium in carrot *in vitro* plants and cell suspension cultures. *Plant Science*, 137(2), 119-129.

- Doğru, A. (2019). Low Temperature Stress and Cold Acclimation in Plants Journal of Osmaniye Korkut Ata University Journal of Nat. & App. Sci. 2:(1), pp 45-52 in Turkish.
- Faraloni, C., Cutino, I., Petruccelli, R., Leva, A. R., Lazzeri, S., & Torzillo, G. (2011). Chlorophyll fluorescence technique as a rapid tool for *in vitro* screening of olive cultivars (*Olea europaea* L.) tolerant to drought stress. Environmental and Experimental Botany, 73, 49-56.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. In Sustainable Agriculture (pp. 153–188). Springer Netherlands.
- Fávero Peixoto-Junior, R., Mara de Andrade, L., dos Santos Brito, M., Macedo Nobile, P., Palma Boer Martins, A., Domingues Carlin, S., ... & Creste, S. (2018). Overexpression of ScMYBAS1 alternative splicing transcripts differentially impacts biomass accumulation and drought tolerance in rice transgenic plants. PloS one, 13(12), e0207534.
- Franco, J. A., Bañón, S., Vicente, M. J., Miralles, J., & Martínez-Sánchez, J. J. (2011). Root development in horticultural plants grown under abiotic stress conditions - a review. In Journal of Horticultural Science and Biotechnology 86:6, pp. 543–556. Headley Brothers Ltd.
- Fu XZ, Ehsan UK, Hu SS, Fan QJ, Liu JH (2011b) Overexpression of the betaine aldehyde dehydrogenase gene from *Atriplex hortensis* enhances salt tolerance in the transgenic trifoliolate orange (*Poncirus trifoliata* L. Raf.). Environ Exp Bot 74C:106–113
- Fu, X. Z., Khan, E. U., Hu, S. S., Fan, Q. J., & Liu, J. H. (2011). Overexpression of the betaine aldehyde dehydrogenase gene from *Atriplex hortensis* enhances salt tolerance in the transgenic trifoliolate orange (*Poncirus trifoliata* L. Raf.). Environmental and Experimental Botany, 74, 106-113.
- Guo, X., Zhang, L., Wang, X., Zhang, M., Xi, Y., Wang, A., & Zhu, J. (2019). Overexpression of *Saussurea involucreata* dehydrin gene SiDHN promotes cold and drought tolerance in transgenic tomato plants. PLoS One, 14(11), e0225090.
- Hadrami, A. E., Daayf, F., & Hadrami, I. E. (2011). *In Vitro* Selection for Abiotic Stress in Date Palm, In: Jain, S., Al-Khayri, J., Johnson, D. (eds) Date Palm Biotechnology. Springer, Dordrecht.
- Hajihashemi, S. and Ehsanpour, A. A. (2013). Influence of Exogenously Applied Paclobutrazol on Some Physiological Traits and Growth of *Stevia rebaudiana* Under *in vitro* Drought Stress, Biologia 68, 414–420.
- Hannachi, S., Werbrouck, S., Bahrini, I., Abdelgadir, A., Siddiqui, H. A., & Van Labeke, M. C. (2021). Obtaining salt stress-tolerant eggplant somaclonal variants from *in vitro* selection. Plants, 10(11), 2539.

- Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., Fujita, M. (2013). Physiological, Biochemical and Molecular Mechanisms of Heat Stress Tolerance in Plants, *Int. J. Mol. Sci.* 2013, 14, 9643-9684.
- Khan, Z., & Upadhyaya, H. (2019). Impact of nanoparticles on abiotic stress responses in plants: an overview. *Nanomaterials in plants, algae and microorganisms*, 305-322.
- Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E., Scharf, K. D. (2007). Complexity of The Heat Stress Response in Plants, *Current Opinion in Plant Biology*, Vol. 10, No. 3, pp. 310–316.
- Kumar, V., AlMomin, S., Al-Shatti, A., Al-Aqeel, H., Al-Salameen, F., Shajan, A. B., & Nair, S. M. (2019). Enhancement of heavy metal tolerance and accumulation efficiency by expressing Arabidopsis ATP sulfurylase gene in alfalfa. *International Journal of Phytoremediation*, 21(11), 1112-1121.
- Legay, S., Guignard, C., Ziebel, J., & Evers, D. (2012). Iron uptake and homeostasis related genes in potato cultivated *in vitro* under iron deficiency and overload. *Plant physiology and biochemistry*, 60, 180-189.
- Li, P., Cai, J., Luo, X. et al. Transformation of wheat *Triticum aestivum* with the HvBADH1 transgene from hullless barley improves salinity-stress tolerance. *Acta Physiol Plant* 41, 155 (2019). <https://doi.org/10.1007/s11738-019-2940-8>
- Liu, H., Gao, Y., Wu, M., Shi, Y., Wang, H., Wu, L., & Xiang, Y. (2020). TCP10, a TCP transcription factor in moso bamboo (*Phyllostachys edulis*), confers drought tolerance to transgenic plants. *Environmental and Experimental Botany*, 172, 104002.
- Lombardi, Lara, Luca Sebastiani, and Claudio Vitagliano. Physiological, biochemical, and molecular effects of *in vitro* induced iron deficiency in peach rootstock Mr. S 2/5. *Journal of plant nutrition* 26, no. 10-11 (2003): 2149-2163.
- Mahmoud, L. M., Dutt, M., Shalan, A. M., El-Kady, M. E., El-Boray, M. S., Shabana, Y. M., & Grosser, J. W. (2020). Silicon nanoparticles mitigate oxidative stress of *in vitro*-derived banana (*Musa acuminata* ‘Grand Nain’) under simulated water deficit or salinity stress. *South African Journal of Botany*, 132, 155-163.
- Maleki, M., Ghorbanpour, M., Nikabadi, S., & Wani, S. H. (2019). *In Vitro* Screening of Crop Plants for Abiotic Stress Tolerance, In: Wani, S. (eds) *Recent Approaches in Omics for Plant Resilience to Climate Change*, pp. 75–91, Springer International Publishing
- Mansinhos, I., Gonçalves, S., Rodríguez-Solana, R., Duarte, H., Ordóñez-Díaz, J. L., Moreno-Rojas, J. M., & Romano, A. (2022). Response of

- Thymus lotocephalus In Vitro Cultures to Drought Stress and Role of Green Extracts in Cosmetics. *Antioxidants*, 11(8), 1475.
- Meher, Shivakrishna, P., Ashok Reddy, K., & Manohar Rao, D. (2018). Effect of PEG-6000 Imposed Drought Stress on RNA Content, Relative Water Content (RWC), and Chlorophyll Content in Peanut Leaves and Roots, *Saudi journal of biological sciences*, Vol. 25, No. 2, pp. 285–289.
- Mehri, H., Ghobadi, C., Baninasab, B., & Ehsanzadeh, P. (2015). Evaluation of some physiological and morphological responses of four Iranian grapevine (*Vitis vinifera* L.) cultivars to drought stress under in vitro conditions. *Journal of Plant Process and Function*, 3(10), 115-126.
- Molinari HBC, Marur CJ, Filho JCB, Kobayashi AK, Pileggi M, Júnior RPL, Pereira LFP, Vieira LGE (2004) Osmotic adjustment in transgenic citrus rootstock Carrizo citrange (*Citrus sinensis* Osb. × *Poncirus trifoliata* L. Raf.) overproducing proline. *Plant Sci* 167:1375–1381
- Montoliu, A., López-Climent, M. F., Arbona, V., Pérez-Clemente, R. M., & Gómez-Cadenas, A. (2009). A novel *in vitro* tissue culture approach to study salt stress responses in citrus. *Plant Growth Regulation*, 59(2), 179-187.
- Munns, R. (2005). Genes and Salt Tolerance: Bringing Them Together, *New Phytologist*, Vol. 167, No. 3, pp. 645–663.
- Parihar, P., Singh, S., Singh, R., Singh, V. P., & Prasad, S. M. (2015). Effect of Salinity Stress on Plants and Its Tolerance Strategies: A Review, *Environmental Science and Pollution Research*, Vol. 22, No. 6, pp. 4056-4075.
- Patakas, A. (2012). Abiotic Stress-Induced Morphological and Anatomical Changes in Plants, In: Ahmad, P., Prasad, M. (eds) *Abiotic Stress Responses in Plants*, pp. 21-39. Springer, New York, NY.
- Pérez-Clemente, R. M. , & Gómez-Cadenas, A. (2012). *In vitro* Tissue Culture, a Tool for the Study and Breeding of Plants Subjected to Abiotic Stress Conditions. In A. Leva, & L. M. R. Rinaldi (Eds.), *Recent Advances in Plant in vitro Culture*.
- Praveen Kumar, G., Mir Hassan Ahmed, S. K., Desai, S., Leo Daniel Amalraj, E., & Rasul, A. (2014). *In Vitro* Screening for Abiotic Stress Tolerance in Potent Biocontrol and Plant Growth Promoting Strains of *Pseudomonas* and *Bacillus* spp., *International Journal of Bacteriology*, Vol. 2014, pp. 1–6.
- Şahin, Ö., Dumanoglu, H., Sarikamis, G., Javadisaber, J., Ergül, A., & Aydemir, B. Ç. (2022). Tolerance of *Pyrus* spp. and *Cydonia oblonga* as pear rootstocks to iron chlorosis determined by in vitro growth, antioxidant and molecular responses. *Scientia Horticulturae*, 296, 110911.

- Sezer, M.M. (2021). Comparison Of the Activities of Some Mobile Genetic Elements (Transposon) Associated with Drought in Some Citrus Rootstocks. Master Thesis, Çukurova University, pages 1-85.in Turkish.
- Shen, X. J., Wang, Y. Y., Zhang, Y. X., Guo, W., Jiao, Y. Q., & Zhou, X. A. (2018). Overexpression of the wild soybean R2R3-MYB transcription factor GsMYB15 enhances resistance to salt stress and *Helicoverpa armigera* in transgenic Arabidopsis. International journal of molecular sciences, 19(12), 3958.
- Shi, J. B., Wang, N., Zhou, H., Xu, Q. H., & Yan, G. T. (2019). The role of gibberellin synthase gene GhGA2ox1 in upland cotton (*Gossypium hirsutum* L.) responses to drought and salt stress. Biotechnology and Applied Biochemistry, 66(3), 298-308.
- Shen, X. J., Wang, Y. Y., Zhang, Y. X., Guo, W., Jiao, Y. Q., & Zhou, X. A. (2018). Overexpression of the wild soybean R2R3-MYB transcription factor GsMYB15 enhances resistance to salt stress and *Helicoverpa armigera* in transgenic Arabidopsis. International journal of molecular sciences, 19(12), 3958.
- Shi, J. B., Wang, N., Zhou, H., Xu, Q. H., & Yan, G. T. (2019). The role of gibberellin synthase gene GhGA2ox1 in upland cotton (*Gossypium hirsutum* L.) responses to drought and salt stress. Biotechnology and Applied Biochemistry, 66(3), 298-308.
- Simsek, O. (2018). Effect of drought stress in *in vitro* and drought related gene expression in Carrizo citrange. Fresenius Environ Bull, 27, 9167-9171.
- Şimşek, Ö., Dönmez, D., & Kaçar, Y. A. (2018). Investigation into performance of some citrus rootstocks in *in vitro* drought stress conditions. Yüzüncü Yil Üniversitesi Journal of Agricultural Sciences, 28(3), 305-310.
- Soetaert, W., Vanhooren, P. T., & Vandamme, E. J. (1999). The Production of Mannitol by Fermentation, Carbohydrate Biotechnology Protocols, pp. 261-275. Humana Press
- Tang, Y., Bao, X., Wang, S., Liu, Y., Tan, J., Yang, M., ... & Yu, X. (2019). A physic nut stress-responsive HD-Zip transcription factor, JcHDZ07, confers enhanced sensitivity to salinity stress in transgenic Arabidopsis. Frontiers in Plant Science, 10, 942.
- Tang, Y., Bao, X., Zhi, Y., Wu, Q., Guo, Y., Yin, X., ... & Liu, K. (2019). Overexpression of a MYB family gene, OsMYB6, increases drought and salinity stress tolerance in transgenic rice. Frontiers in plant science, 10, 168.
- Tester, M., & Davenport, R. (2003). Sodium Tolerance Sodium Transport in Higher Plants, Annals of Botany, Vol. 91, No. 5, pp. 503–527

- Thudi, M., Palakurthi, R., Schnable, J. C., Chitikineni, A., Dreisigacker, S., Mace, E., Srivastava, R. K., Satyavathi, C. T., Odeny, D., Tiwari, V. K., Lam, H., Hong, Y. B., Singh, V. K., Li, G., Xu, Y., Chen, X., Kaila, S., Nguyen, H., Sivasankar, S., Jackson, S. A., Close, T. J., Shubo, W., & Varshney, R. K. (2021). Genomic Resources in Plant Breeding for Sustainable Agriculture, *Journal of Plant Physiology*, Vol. 257, No. 153351, pp 1-18
- Turhan, Ş. (2017). The Effect of Irrigation Investments on the Usage of Agricultural Areas in Turkey. *Turkish Journal of Agricultural Economics*, 23 (2), 157-163 in Turkish.
- Yang, Y., Yu, T. F., Ma, J., Chen, J., Zhou, Y. B., Chen, M., ... & Xu, Z. S. (2020). The soybean bZIP transcription factor gene GmbZIP2 confers drought and salt resistances in transgenic plants. *International journal of molecular sciences*, 21(2), 670.
- Yao, W., Zhao, K., Cheng, Z., Li, X., Zhou, B., & Jiang, T. (2018). Transcriptome analysis of poplar under salt stress and over-expression of transcription factor NAC57 gene confers salt tolerance in transgenic *Arabidopsis*. *Frontiers in plant science*, 9, 1121.
- Yarra, R., He, S.J., Abbagani, S. et al. (2012). Overexpression of a wheat Na<sup>+</sup>/H<sup>+</sup> antiporter gene (TaNHX2) enhances tolerance to salt stress in transgenic tomato plants (*Solanum lycopersicum* L.). *Plant Cell Tiss Organ Cult* 111, 49–57 <https://doi.org/10.1007/s11240-012-0169-y>
- Wang, C. T., Ru, J. N., Liu, Y. W., Li, M., Zhao, D., Yang, J. F., ... & Xu, Z. S. (2018). Maize WRKY transcription factor ZmWRKY106 confers drought and heat tolerance in transgenic plants. *International Journal of Molecular Sciences*, 19(10), 3046.
- Wijewardene, I., Mishra, N., Sun, L., Smith, J., Zhu, X., Payton, P., ... & Zhang, H. (2020). Improving drought-, salinity-, and heat-tolerance in transgenic plants by co-overexpressing *Arabidopsis* vacuolar pyrophosphatase gene AVP1 and *Larrea* Rubisco activase gene RCA. *Plant Science*, 296, 110499.
- Wong WA, Li GG, Ning W, Xu ZF, Hsiao WLW, Zhang LY, Li N (2001) Repression of chilling-induced ACC accumulation in transgenic citrus by over-production of antisense 1-aminocyclopropane-1-carboxylate synthase RNA. *Plant Sci* 161:969–977
- Wu, J., Jiang, Y., Liang, Y., Chen, L., Chen, W., & Cheng, B. (2019). Expression of the maize MYB transcription factor ZmMYB3R enhances drought and salt stress tolerance in transgenic plants. *Plant physiology and biochemistry*, 137, 179-188.
- Zaki, H. E., & Yokoi, S. (2016). A comparative in vitro study of salt tolerance in cultivated tomato and related wild species. *Plant Biotechnology*, 16-1006.

- Zekai, E., Açar, E., Dönmez, D., Şimşek, Ö., & Aka Kaçar, Y. (2022). In vitro drought stress and drought-related gene expression in banana. *Molecular Biology Reports*, 1-7.
- Zhang, K., Cui, H., Cao, S., Yan, L., Li, M., & Sun, Y. (2019). Overexpression of CrCOMT from *Carex rigescens* increases salt stress and modulates melatonin synthesis in *Arabidopsis thaliana*. *Plant Cell Reports*, 38(12), 1501-1514.
- Zhang, L., Guo, X., Zhang, Z., Wang, A., & Zhu, J. (2021). Cold-regulated gene LeCOR413PM2 confers cold stress tolerance in tomato plants. *Gene*, 764, 145097.

## CHAPTER 13

### SUSTAINABILITY IN SOIL MAINTANANCE BY CORRECT PRACTICES AND FARMER'S AWARENESS

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## **1.1. INTRODUCTION**

In a world that is changing rapidly, can anything be stable? What do we want to maintain? How can we implement such a vague objective? Is it too late? According to the contradictions and questions a net look at our present food production system and correct investigation of the future should be necessary. The term "sustainable agriculture" has provided "discussion cases," a request of direction, and an urgency, that has created excitement and innovative thinking in the agricultural world.

The word "sustain," as a Latin *sustinere* word (*sus-*, from below and *tenere*, to hold), means to the existence or maintain, long-term support or permanence. "Sustainable agriculture" was passed by Congress in the 1990 "Farm Bill" (Food, Agriculture, Conservation, and Trade Act of 1990 (FACTA)). According to this law, the term "sustainable agriculture" means an integrated system of plant and animal production methods that have a specific usage in the long term. Among these applications are providing human food and fiber needs and increasing the quality of soil and environment and natural resources that the agricultural economy depends on. Maintaining the economic survival of farm operations and improving the quality of life of farmers and human society is another application of sustainable agriculture.

The biggest achievement in agriculture will be to achieve the desired increase in production by reducing negative environmental conditions. This is possible only by implementing sustainable methods and sustainable solutions in agriculture. The compatibility and permanence of agricultural activities with the environment is very important in order to contribute to the sustainability of ecology. There is many definitions and explanations about sustainable agriculture.

Sustainable agricultural systems and methods improve the protection of the environment and natural resources of agriculture to ensure the production

of sufficient and quality food. In general, sustainable agricultural methods are affordable and suitable for the world's rapidly growing population. To be completely self-sufficient for sustainable agriculture is not a requirement. Long-term stability and efficiency is necessary.

To create awareness about sustainable practices, we must first review the concept of agriculture in detail. Questions such as: How to produce to meet the growing needs for agriculture? What methods should be implemented to access sufficient products without harming nature? At first should be answered the questions.

Sustainable agriculture increases soil fertility and reduces the harmful effects of agricultural operations on climate, soil, water, environment and human health. It also reduces the use of non-renewable resources and inputs of petroleum products and uses more renewable resources for production. Sustainable agriculture is based on the needs, knowledge, skills and socio-cultural values of local people.

Sustainable agriculture is based on human goals and on identifying the long-term effects of human activities or other animal and plant species on the environment. Sustainable and protective agricultural systems reduce environmental degradation, increase agricultural efficiency, They improve economic development in the short and long term and stabilize rural communities and quality of life. Sustainable agriculture does not mean a decrease in production or the poverty of poor farmers. Rather, it causes high yield and agricultural profits and prevents the weakening of agricultural resources. Discussion about sustainable agriculture should be continue by the different groups as farmers, economics, sociologists and researchers.

This chapter aim is about the different concepts of sustainable agriculture. How the farmers can perform the methods of sustainbale agriculture correctly. Sustainable agriculture development should be

according to the universal principles and all of aspects such as soil, water, natural resources, environment and human request should be planned to perform its ways. This chapter is a small step to receive this goal.

## **1.2. PRINCIPLES OF SUSTAINABLE AGRICULTURE**

**Soil development and protection:** Soil is an essential factor for producing good and healthy products. The soil should be enriched with natural fertilizers such as organic and green manures and compost. Natural fertilizers are less toxic than chemical fertilizers and provide healthier soil resources for plants and people and do not cause to the air and water pollution (Pretty, 2008). **Necessity of protecting water and water resources:** Agricultural work requires water. In dry areas, growing plants suitable for the region's ecology is a good way to conserve water. Green manure and mulch help to retain water in the soil. Preventing water flow by contour barriers is another way to protect water. Using drip irrigation instead of traditional methods of irrigation is a basic measure to protect the wastage of water resources (Aydemir, 2018). **Control of pests and plant diseases:** Instead of chemical control, control with organic farming methods and natural conservation management should be done to control pests, diseases and weeds. Techniques such as selection of resistant cultivars, microbiological methods and use of organic materials can be useful. **Planting different agricultural products:** This type of planting is called crop rotation. According to the characteristics of different crops, rotation or cultivation of several crops for 3 to 6 years prevents the emergence of plant diseases and pests. Therefore, soil nutrients are preserved and healthy and higher quality crops are produced. **Start with small changes:** Agricultural techniques take a long time to develop. But new methods are not always successful. New ideas should be tested on a small scale and implemented later when these methods are proven to be successful (Smitha et al., 2007).

### **1.3. WHY SUSTAINABLE AGRICULTURE IS IMPORTANT?**

The world population is growing rapidly. There are countries like Asian countries that have a population of billions and in Europe and the Americas the population will soon reach billions. This shows a serious need for food in the future. Industrial agriculture implements sustainable methods with the aim of ensuring the access of the entire world population to basic needs and food in the coming years (Foley et al., 2011).

The benefits of sustainable agriculture in such a world with an increasing population can be as follows:

- a) By using sustainable agricultural methods, more than one crop can be planted in small areas and produced with high efficiency.
- b) It has positive effects on the ecosystem. The habitat of animals will have quality soils suitable for animal life and it will increase agricultural production.
- c) Long-term usage and increase in soil productivity is possible with appropriate and correct fertilization.

### **1.4. SUSTAINABLE AGRICULTURE PRACTICES**

Over many years and evaluations, some common methods of sustainable agriculture are shown in Figure 1.



**Figure 1:** Common sustainable agricultural practices (Ussec, 2019).

#### **1.4.1. Precision Chemical Application**

It includes mechanical and biological controls to reduce the use of pesticides and control pests, plant diseases and weeds. In this method, prevention is done according to the needs of the farm, and timing is very important in this method. By using appropriate inputs, we try to obtain maximum inputs and productivity in the farm (Garnett et al., 2013).

#### **1.4.2. Conservation Reserve Program**

The Farm Service Agency administers Program CRP, a land conservation program. Signing a contract with farmers in this program increases the incentive to produce environmentally sensitive agricultural products and improve the health and quality of the environment for the farmers. Recovery of plant species in the region in order to improve water quality, prevent soil erosion and reduce the loss of wildlife areas is one of the long-term goals of the program (Krall, 2019).

### **1.4.3. Terraces**

In sloping lands, if the land is designed in the form of steps to carry out agricultural work, and these steps rely on the walls, this design is called a terrace. This system makes agriculture possible in sloping lands (Garnett et al., 2013).

### **1.4.4. Scouting**

Scouting is doing close observation in the field. This means that the farmer is obliged to carry out the necessary investigations in different parts of the farm and identify how to develop different areas of the farm and make the necessary changes (USDA, 2019).

### **1.4.5. Cover Crops**

In the periods after planting and when the soil is bare or when the field is under cultivation, cover plants can be planted between the main rows of plants. These products prevent soil erosion, improve soil nutrients, inhibit weed growth and thus increase soil health by reducing the use of herbicides in the field (Opio et al., 2013).

### **1.4.6. Crop Rotation/Diversity**

If the crops planted in the field change every year, this process is called crop rotation. With this method, different products are used in different parts of the farm every year, which prevents the spread of pests and plant diseases in the fields (Giller et al., 2015).

### **1.4.7. No-till/Conservation Tillage**

The negative effects of intensive or traditional agriculture include physical and chemical degradation of the soil, loss of organic matter, reduction of biological activity in the soil and, as a result, reduction of crop production. The sustainable farming method expresses a sustainable farming system to

avoid these negative effects. In this way, sustainable agriculture includes three basic laws of agriculture without soil, continuous surface of soil covered with plant residues and crop rotation (Liu and Yang, 2007).

#### **1.4.8. Precision Nutrient Management**

Product yield can be increased by 50% due to soil fertilization. Agricultural fertilizers account for approximately 10 to 15 percent of agricultural input costs. The time and method of application of agricultural fertilizers to provide essential nutrients (nitrogen, phosphorus, potassium) or to provide micro and macro nutrients in the soil or in the form of leaf spray is very important. To determine the fertilization time, data such as weather and climate conditions, soil characteristics and plant species are also important (Zhao et al., 2019).

#### **1.4.9. Reducing Fuel Use**

Mechanical machines that reduce the need for labor in agriculture generally use fossil fuels. Today, the use of fossil fuel energy is not economical for farmers. The production of agricultural fertilizers and agricultural processes is not possible without the use of fuel, but if green energy can be replaced by fossil fuels, it will have positive effects both economically and environmentally (Pittelkow et al., 2015).

#### **1.4.10. Irrigation**

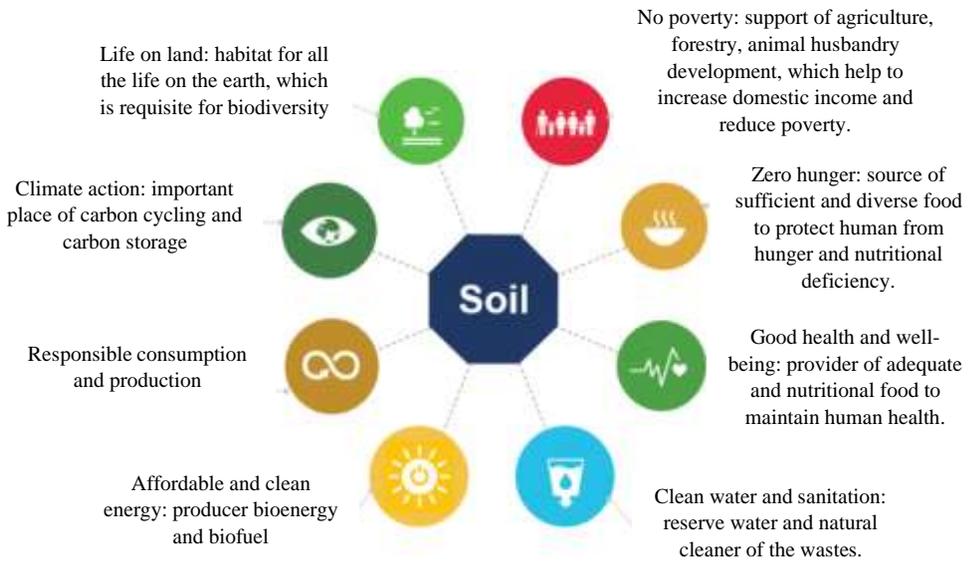
The use of different parameters such as soil moisture, effective rate of rainfall and evaporation and transpiration to determine the appropriate amount of irrigation water and also determine the correct time of irrigation using climatic data, weather forecast and real-time weather data will cause effective irrigation. Protection of limited water resources causes effective and economic irrigation and prevents negative environmental and agricultural effects caused

by leaching, salinity and fungal diseases caused by high humidity (Bos et al., 2014).

### 1.4.11. Water Storage Ponds

Agricultural ponds are important structures of water resources that collect water from small sources. By storing water in these structures, they provide the use of large water flows when needed and help to regulate water distribution.

### 1.4.12. Sustainable Development Goals



**Figure 2:** The relationship between soil and Sustainable Development Goals (Deyi et al., 2020).

## 1.5. SUSTAINABLE SOIL MANAGEMENT

Today, 33% of the world's soils are degraded at moderate and high levels. Erosion, salinization, acidification, chemical pollution caused by land degradation, population growth, urban expansion and intensive cultivation

cause soil degradation to continue increasingly. Soil management is sustainable soil management if the support, supply, regulation and cultural services provided by the soil can be sustained or enhanced without harming the soil functions and biodiversity that make these services possible. Investments in sustainable soil management should be increased and local soil-friendly practices should be encouraged. An ecosystem-based holistic management style that takes into account the unique structure of the soil should be supported (Batalla et al., 2015).

An international framework for assessing sustainable land management definitions by FAO Sustainable land management combines socio-economic principles with environmentally sensitive technologies, policies and activities. For the implementation of sustainable land management, five main pillars have been identified as efficiency, security, protection, vitality and acceptability and implementation and findings of the SLM regulation. The specific features of each goal are explained as follows:

**Efficiency:** The return obtained from sustainable land management does not only include financial profit. It also includes other benefits including conservation, purposes and provides soil and water health (Buratti et al., 2017).

**Security:** Management models create a balance between land use and environmental conditions and reduce production risks. This model also reduces the possibility of business risk.

**Conservation:** Future generations are in dire need of soil and water resources, so these resources must be protected for future generations. In local scales, in addition to water and soil resources, genetic, plant and animal species should also be protected.

**Vitality:** The applied uses must be in accordance with the local conditions, otherwise the applied uses at the local scale will not be continuous and will not last.

**Acceptability:** Land use methods must be acceptable from the point of view of the people, otherwise it will fail over time. Of course, some of the land uses that are affected by social or economic demands are not completely clear and more investigations should be done.

## **1.6.MAINTAINING AND IMPROVING SOIL PRODUCTIVITY**

### **1.6.1.Managing Soil Nutrients**

In agriculture, the timely use of nutrients with appropriate and correct methods leads to healthy plant nutrition. Various factors contribute to the proper management of plant nutrition. For example, if we can reduce the loss of nutrients due to washing, denitrification, evaporation and surface runoff, the efficiency of fertilizer use will increase. Some fertilizers are not suitable for use in this technique, such as nitrogen, which can be released into a gaseous state, or phosphorus and potassium, which are converted into non-volatile forms. In general, 50% of the nitrogen introduced into the soil is wasted in different ways, and almost 90% of the consumed phosphorus cannot be absorbed by plants and remains in the soil in a fixed state. In different methods of working on the soil, such as minimum tillage, conventional tillage, conservation tillage and no-till farming systems, there is a significant difference between the quality of soil organic matter and the availability of nutrients (Gaj, 2011). With some soil characteristics such as total organic carbon, volumetric weight, aggregate strength, usable moisture, pH and EC, soil quality can be recognized. Agricultural systems also affect soil water holding capacity, soil water movement in soil, soil compaction and soil

temperature. For this reason, soil management has a significant impact on the efficiency of fertilizer use. The types of applied fertilizers in farms, which include chemical fertilizers and organic fertilizers, the applied dose of fertilizers and the time and method of using fertilizers, as well as the irrigation method, are among the subsets of soil management. Also, the method of using fertilizers is important from an economic point of view (Rae, 2019). By managing soil elements, it is possible to increase the efficiency of fertilizer use and use less fertilizer on smaller scales. With this method, the wastage of some nutritional elements such as nitrogen is prevented, and plants can use fertilizers in the long term, and the efficiency of fertilizers increases. Another factor that affects the rate of fertilizer consumption is soil analysis and soil sampling technique. Because the physical and chemical properties of soil are very variable in different agricultural areas, it should be done in different parts regionally and even at the farm level, soil characteristics to be analyzed. In this way, according to the test results, the fertilizer should be used appropriately (Herliana et al., 2019).

### **1.6.2. Managing Soil Physical Conditions**

Soil micro-organisms continue their biological activity in the soil under vegetation and live and feed in the roots of plants or plant residues in the soil. They made air pore into the soil and improve the soil physical properties. Rainfall, human and animal activities, and the work of machines have a great impact on soil compaction, followed by vegetation compaction. If we can provide a certain density in the sabbath soil for the growth of plant roots, we will increase the ability of plants to retain the water needed to survive. The compacted soil dries out and as a result creates a crust on the soil surfaces. For this reason, the rate of water penetration in the soil decreases and may cause water to flow from the soil surface and soil erosion. Population growth increases the need for food in communities (Jankowiak et al., 2013). To meet

the nutritional needs, if more pressure is applied to the soil, and on the other hand, soil and nutrients are not managed, the fertility of the soil will be lost and the structure of the soil will deteriorate. Management of soil physical properties includes the protection of soil structure necessary for the production of agricultural products, as well as the use of agricultural techniques and processing techniques to increase the long-term performance of the soil. Under the conditions of soil management, more environmentally friendly production conditions will be created and healthier, more economical and higher quality production will be provided. Vegetation is also important as weed control and leads to a reduction in the use of herbicides. The movement of agricultural machinery causes compression on the soil surface, especially in the deeper layers, and destroys the soil structure. In soil compaction, the soil grains are destroyed and the pores between the soil particles are reduced. Reducing the density of soil pores reduces soil ventilation, water drainage and water penetration into deep layers and causes surface flow in rainy conditions. Soil compaction also makes seed germination more difficult, limits plant root growth, affects soil biodiversity, and causes surface flaking (Kata and Kusz, 2015).

Some of the cases that be attended for the protection of soil physical structure can be listed as follows:

- a) The number of agricultural machines and the frequency of these machines in the farm should be reduced and unnecessary operations in the farm should be avoided.
- b) The machines should be selected based on the field work and also consider the soil properties. The tire pressure should be adjusted to reduce the pressure on the soil and reduce it if necessary.

- c) Some agricultural practices that increase soil organic matter and improve soil structure, such as soil aeration, heat transfer, and root growth, should be increased.
- d) In grazing systems, the intensity and timing of grazing should be well planned.

### **1.6.3. Water Management**

If enough moisture is maintained in the root zone of the soil, the growth of the plant is guaranteed to some extent. Rainfall is a natural source of water supply, if rainfall is not enough, the water needed for plant growth must be provided through irrigation. If the soil moisture in the root zone is not provided for the plant, the yield of the plant will decrease. Social, physical, economic and ecological concepts are included in the concept of sustainability of water resources. In sustainable water management, water needs of future generations, drinking and usage water, irrigation water, protection of industrial and recreational water and ecosystem protection services should be considered (Tilman et al., 2002).

Continuous control of the irrigation system is essential. Irrigation pumps must work with good performance and even if they are controlled once, measuring the amount of water and uniform distribution of water is also essential.

The water content of the plant should determine the time and amount of irrigation to ensure the optimal use of irrigation water. Irrigation should not be done in the middle of the day and in windy weather, irrigation at night has a good efficiency, and if possible, it is better to use the drip irrigation method. Water pollution, pollution of water sources and drainage channels should be avoided. If irrigation is done according to the principles of irrigation and the irrigation method is perpendicular to the slope of the land, it can be ensured that the irrigation efficiency is appropriate and the irrigation water will have a

good penetration into the soil. According to the quantity of water and the distance of water sources, it should be planned for agricultural production (Jena et al., 2017).

Today, agricultural technology also offers new methods. By using UAV and sensor technologies, the required data can be collected and effective developments can be made in agricultural methods, including irrigation methods. Based on this:

- Special sensors for determining soil moisture can determine soil water potential.
- Determining soil moisture and plant products using thermal images obtained from drones.
- Multispectral cameras can measure soil and plant nitrogen deficiency.
- According to weather data and weather forecasts, a precise irrigation program can be created with variable amount of irrigation water and irrigation time.
- According to the optimal timing in different fields, the use of agricultural inputs can be determined or changed based on irrigation water.

#### **1.6.4.Pests And Diseases Management**

Under integrated pest management (IPM), which is one of the effective methods used in modern agriculture, all plant protection methods in the program can be considered. Schlumb's IPM is the measures that take the environmental protection of human health and production and are applied in the lowest economic conditions. IPM takes necessary measures to prevent harmful microorganisms and reduce chemical control. Also, in the determination of crop rotation, the use of appropriate breeding methods, the

use of tolerant cultivars and certified seeds, the use of balanced fertilization, liming, and the appropriate applications of irrigation or drainage, he suggests several methods in agricultural science.

### **1.6.5. Cover Crop And Rotation**

To protect the soil, temperature, humidity or light at the desired level, controlling pests and weeds of cover plants is effective and this will have positive effects on the agricultural production. Planting cover plants also reduces the growth of weeds (Lubell et al., 2011). The purpose of producing and using cover plants is different. Some plants such as clover, vetiver, sorghum, oats, rye, sorghum can be planted as cover plants in addition to their main production purpose. Planting cereals can be used to control weeds, and planting legumes can be used to provide soil nitrogen for crops. The balance between the production cost of cover plants and the benefits obtained from the cultivation of these plants must be observed and considered the most basic point in the production of these plants. The system of cover plants should be such that it reduces the input cost of agricultural inputs and increases the efficiency of the crop. Cover plants protect the plant mass of the area and increase the organic matter of the soil. Cover plants increase soil aeration and encourage plant root growth. In this way, the surface flow of water on the soil is reduced and soil erosion is reduced. In addition, cover plants increase soil microorganisms and earthworms and improve nutrient cycling and soil structure. On the other hand, it is possible to reduce soil tillage, increase soil organic matter, benefit from different depths of nutrients, protect soil moisture, increase soil water holding capacity and control weeds. For this reason, cover cropping and crop rotation in sustainable agriculture is one of the important applications to reduce production inputs and create economic agriculture (Karami and Mansoorabadi, 2007).

## **1.7.FARMER AWARENESS**

One of the basic aspects of sustainable agriculture is for the farmer to be aware of the risks associated with agricultural production and to have enough information about how to produce in a sustainable way. In addition, the farmer must believe in the sustainable production method and have the motivation to implement sustainable agriculture so that he can implement and continue this method in his lands with interest. If it is possible to increase the awareness of the farmers of a region about the environmental effects and yield as well as the economic aspect of agricultural systems, this issue will lead to the movement towards alternative agricultural systems (such as sustainable or organic agriculture). Farmers are usually aware of the importance of sustainable agriculture and limiting the use of chemical fertilizers. The only factor that causes traffic in farmers to implement these methods is the fear of low yields of agricultural and garden crops. Also, farmers have little information about inputs. In recent years, the increase in the price of chemical inputs has encouraged farmers to find alternative inputs. For farmers, the most important factor is the balance between input and output along with the profit earned. While they know the importance of the aspects of genetic preservation and plant and animal species and environmental protection. For this reason, in addition to little knowledge and awareness, cultural activities should be done for farmers. In the research conducted, it has been shown that 65% of farmers had an average attitude towards sustainable farming methods, while 21.1% of farmers had a weak attitude and only 13.3% of farmers had a positive attitude towards sustainable farming methods (Ghosh and Hasan, 2013). The level of education of the farmer, the age of the farmer, the size or smallness of the farm, the annual income and the financial situation of the farmer, the knowledge and motivation of the farmer to find alternative methods have a significant positive relationship with the willingness of farmers towards

sustainable agriculture practices. Information to farmers will be successful when it is local and regional and is done according to the moral, environmental and social values specific to the region. The methods that farmers use in their lands are generally due to the expertise, ethics and experience of farmers. In order to implement sustainable agricultural methods, the capacity to accept farmers should be increased and enough information should be given to farmers about all aspects of this method so that the farmer can implement these methods with a general view of the system. Farmers should know that implementing sustainable farming methods will increase or decrease their yield (Story and Forsyth, 2008). The desire to implement sustainable practices should be based on motivation and knowledge rather than following rules. In addition to the social benefits that sustainable farming methods bring, food safety ranks highest among the benefits of implementing this method. Justifying and introducing the principles of sustainable agriculture in the farm will produce products with better performance and thus improve the profitability of the farm, which has a positive effect on the sustainable economic pillar of the farmer. One of the common reasons for incorrect and inappropriate production decisions that negatively affect the natural environment is the farmer's insufficient understanding of the concept of sustainability. Over the past few decades, various concepts have been applied in research and policy to encourage farmers to implement sustainable agricultural practices. These researches are usually carried out by agricultural research departments or the Ministry of Agriculture, but private sectors play a significant role in transferring these researches and policies to farmers. Because the engineers of the private sector are more in direct contact with the farmers and are more familiar with the regional culture of the villages. But it should be noted that government aid (such as the provision of agricultural

inputs or the percentage of payment for input inputs) is also effective in farmers' decisions and encouraging them (Hyland et al., 2016).

## **1.8. CONCLUSION**

It is not possible to manage agricultural areas with only traditional methods and assessment of soil characteristics, and this is not enough for optimal soil utilization. In determining a more sustainable agricultural system, the physical and chemical structure and quality of the soil must be accurately evaluated, and the factors affecting the soil must also be examined. In this way, a successful sustainable agricultural system can be created in the region. Today, technology can be used to implement sustainable agricultural methods. Agriculture 4.0 with more economic costs and better performances can take valuable measures in line with the implementation of sustainable agriculture. In addition to this, intelligent systems are environmentally friendly and cause less damage to the environment. These intelligent systems can be used in all aspects of sustainable agriculture, such as soil and plant analysis. With the use of smart systems, accurate and real-time information such as the type and amount of fertilizer can be obtained

The lands of different regions were obtained by using intelligent systems of weather conditions, mineral needs of plants, irrigation time, soil condition, estimation of harvest time. Workload and cost are reduced with machines working simultaneously. The farmer can view the entire farm using a tablet or mobile phone and get information, and by reducing the labor force, new efficient, more economical, high-quality and natural production facilities are created. All these intelligent systems can provide serious assistance for the implementation of sustainable agricultural methods and make the implementation of sustainable methods easier and more economical. In this way, the farmer will be more encouraged to implement sustainable methods. In order to implement these works, it is necessary to increase the farmer's

understanding of the subject and help the farmer in the initial stages of implementing sustainable agricultural methods step by step so that the farmer can follow these methods with more confidence.

## 1.9. REFERENCES

- Aydemir, G, Oguz O. (2018). Sustainable Agriculture (in Turkish). WWF-Türkiye (Accessed on 30 March 2018). Available at: <http://www.wwf.org.tr>.
- Batalla, I., Knudsen, M.T., Mogensen, L., Hierro, O., Del Pinto, M., Hermansen, J.E. (2015). Carbon footprint of milk from sheep farming systems in Northern Spain including soil carbon sequestration in grasslands. *Journal of Cleaner Production*, 104: 121-129.
- Bos, J.F.F.P., De Haan, J., Sukkel, W., Schils, R.L.M. (2014). Energy use and greenhouse gas emissions in organic and conventional farming systems in the Netherlands. NJAS Wageningen. *Journal of Life Science*, 68: 61-70.
- Buratti, C., Fantozzi, F., Barbanera, M., Lascaro, E., Chiorri, M., Cecchini, L. (2017). Carbon footprint of conventional and organic beef production systems: An Italian case study. *Science of the Total Environment*, 576: 129-137.
- Deyi, H., Nanthi, S.B., Daniel, C.W., Tsang, Mary B.K., O'Connor, D. (2020). Sustainable soil use and management: An interdisciplinary and systematic approach. *Science of the Total Environment*, 729: 1-12.
- Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Zaks, D.P.M. (2011). Solutions for a cultivated planet. *Nature*, 478: 337-342.
- Gaj, R. (2011). Effect of Diversified Phosphorus and Potassium Fertilization on Plant Nutrition at the Stage of Initial Main Shoot Development and the Yield and Oil Content in the Seeds of Winter Rapeseed. *Acta Scientiarum Polonorum Agricultura*, 10: 57-68.
- Garnett, T., Appleby, M.C., Balmford, A., Bateman, I.J., Benton, T.G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., Fraser, D., (2013). Sustainable intensification in agriculture: Premises and policies. *Science*, 341: 33-34.
- Ghosh, M.K., Hasan, S.S. (2013). Farmers' Attitude towards Sustainable Agricultural Practices. *Bangladesh Research Public Journal*, 8: 227-234.
- Giller, K.E., Andersson, J.A., Corbeels, M., Kirkegaard, J., Mortensen, D., Erenstein, O. (2015). Beyond conservation agriculture. *Frontiers in Plant Science*, 6:870.
- Herliana, O., Harjoso, T., Anwar, A.H.S., Fauzi, A. (2019). The Effect of Rhizobium and N Fertilizer on Growth and Yield of Black Soybean (*Glycine max* (L) Merril). *IOP Conference Series Earth and Environmental Science*, 255.
- Hyland, J.J., Jones, D.L., Parkhill, K.A., Barnes, A.P., Williams, A.P. (2016). Farmers' perceptions of climate change: Identifying types. *Agriculture Human Values Journal*, 33: 323-339.
- Jankowiak, J., Bienkowski, J., Holka, M., Dabrowicz, R., Chudzinski, Z. (2013). Chemical Plant Protection and Its Environmental Impact Under Conditions of Resowing after Frost Killing of Winter Crops. *Infrastructure and Ecology of Rural Areas*, 2: 45-59.
- Jena, A., Biswas, P., Saha, H. (2017). Advanced Farming Systems in Aquaculture: Strategies to Enhance the Production. *Innovative Farming*, 2: 84-89.
- Karami, E., Mansoorabadi, A. (2007). Sustainable agricultural attitudes and behaviors: A gender analysis of Iranian farmers. *Environment Development Sustainability*, 10: 883-898.

- Kata, R., Kusz, D. (2015). Barriers to the Implementation of Instruments Assisting Sustainable Development of Agriculture. *Scientific Paper Series Management Economic Engineering Agriculture Rural Development*, 15: 239-248.
- Krall, S. (2015). What is sustainable agriculture? Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH. (Accessed on 05 June 2019). Available at: <http://www.giz.de/nachhaltige-landwirtschaft>
- Liu, G., Yang, X. (2007). Spatial Variability Analysis of Soil Properties within a Field. In: *Computer and Computing Technologies in Agriculture*. Boston, MA: Springer; Vol. II. p.1341-1344.
- Lubell, M., Hillis, V., Ho\_man, M. (2011). Innovation, cooperation, and the perceived benefits and costs of sustainable agriculture practices. *Ecology Society*, 16, 23.
- Opio, C., Gerber, P., Mottet, A., Falcucci, A., Tempio, G., MacLeod, M., Vellinga, T., Henderson, B., Steinfeld, H. (2013). Greenhouse Gas Emissions from Ruminant Supply Chains—A Global Life Cycle Assessment; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy.
- Pittelkow, C.M., Liang, X., Linquist, B.A., van Groenigen, K.J., Lee, J., Lundy, M.E. (2015). Productivity limits and potentials of the principles of conservation agriculture. *Nature*, 517: 365-368.
- Pretty, J. (2008). Agricultural sustainability: Concepts, principles and evidence. *Philosophical Transactions of the Royal Society B*, 363: 447-465.
- Rae, Z.H.A. (2019). On Evaluating the Effect of Soil Treatment and Fertilizer on the Cultivation of Grain Crops. *Journal of agriculture and aquaculture*, 1: 1-5.
- Smitha, D.R., Owensb, P.R., Leytemc, A.B., Warnemuendea, E.A. (2007). Nutrient losses from manure and fertilizer applications as impacted by time to first runoff event. *Environmental Pollution*, 147(1):131-137.
- Story, P.A., Forsyth, D.R. (2008). Watershed conservation and preservation: Environmental engagement as helping behavior. *Journal of Environment and Psychology*, 28: 305-317.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature*, 418: 671-677.
- USDA. (2019). Conservation Reserve Program. United States Department of Agriculture Farm Service Agency. (Accessed on 05 June 2019). Available at: <https://www.fsa.usda.gov/programs-and-services/conservation-rograms>.
- Ussec. (2019). A Framework for Sustainable Farming. (Accessed on 05 June 2019). Available at: <https://ussec.org/resources/soybean-growth-cycle>.
- Zhao, H., Li, X., Jiang, Y. (2019). Response of nitrogen losses to excessive nitrogen fertilizer application in intensive greenhouse vegetable production. *Sustainability*, 11:1513.



## CHAPTER 14

### USAGE OF PERVIOUS CONCRETE FOR SUSTAINABLE AGRICULTURAL MANAGEMENT

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## INTRODUCTION

Protection of the agricultural lands from floods is an important issue in order to sustainable agriculture. Since land use is one of the factors that directly affect floods, construction materials with high permeability (leakage potential) must be used. These materials shows that they can be used as a useful element in reducing the effect of floods. Moreover, harvested water from permeable concrete can also be used in agricultural areas for irrigation purpose.

Global warming is a concept that defines the increase in the use of fossil fuels with the industrial revolution, the accumulation of gases (carbon dioxide, methane, hydrofluorocarbons, etc.) released into the atmosphere, affecting the properties of the atmosphere, creating a greenhouse effect and warming the earth (Tatar, 2018). Climate change negatively affects all creatures by disrupting the world ecosystem, with the reduction of agricultural and forest areas due to the improper land use (Canlı, 2010; Tatar, 2018).

Climate change causes natural disasters such as drought, irregular precipitation and forest fires. This situation makes it difficult to access water and food on Earth, facilitates the emergence of epidemics, the decrease in species due to damage to plant and animal ecosystems, and the extinction of some species (Canlı, 2010; Uysal, 2022).

Global warming and climate changes affect major sectors such as agriculture, food production, fishery industry, livestock, forestry, foreign trade, tourism, health, construction, logistics and finance-insurance. The agricultural sector is also one of the sectors that are affected by this situation and it is important in terms of sustaining people's lives. For this reason, it has an important place in economies with its share in national income, employment, foreign trade, agriculture-based industry, consumption expenditures (Bayraç and Doğan, 2016).

Although the agricultural sector is an area where technology is used, it is also a sector intertwined with nature (Bayraç and Doğan, 2016). Overheating in the atmosphere due to global warming triggers periodic drought by causing irregular precipitation and evaporation to increase, and beneficial precipitation to decrease. (Küçükklavuz, 2009; Çabuk, 2011; Traşçı and Erdoğan, 2022). This situation causes global climate change, decreases the moisture content in the soil with the resulting warming, and thus causes a decrease in the cultivated agricultural land (Aksay et al., 2005; Traşçı and Erdoğan, 2022).

Precipitation changes are an important parameter in agricultural drought and the drought can be determined by monitoring the changes in precipitation for many years. Areas with a precipitation variation coefficient of more than 25% are defined as arid and semi-arid areas. Since the amount of water may decrease with the increase of this coefficient, this ratio has vital importance for agricultural areas. For this reason, in areas where the coefficient of variability is high, water consumption should be done less and consciously. The variability coefficient of the annual precipitation in our country is more than 20%. This situation shows that our country is poor in terms of water (Türkeş, 1990; Ölgen, 2010). Therefore, the researchers have tended to use useful building materials in agricultural building in order to prevent drought by using water with maximum efficiency. This study aims to develop a perspective on permeable concrete. Because permeable concrete is very useful in collecting the precipitation without causing flooding, collecting it in water tanks and evaluating it in agricultural irrigation systems.

## **PERVIOUS CONCRETE**

When water comes into contact with porous materials, the materials let water to pass from one point to another. The porosity and water absorption rate of these materials are higher than other materials. Such materials are also

called hygroscopic materials. Hygroscopic materials are wettable materials, and after contact with water, they fill the spaces in the material with the effect of capillarity. It works with the principle that water is absorbed by the material due to the pressure difference (Gönül and Çelebi, 2003). Permeable concrete is also a building material that can be wetted and can transfer water from one point to another. In that, permeable concrete, which has a spongy structure, is a special type of concrete with a small amount of thin aggregate (Figure 1). These gaps in the permeable concrete facilitate the flow of water (Boğa, 2015).



**Figure 1.** Pervious concrete (Anonim, 2022a)

When permeable concrete is considered technically, it generally consists of 15-30% gap, 0.25-0.35 water/cement ratio, 3-30 MPa compressive strength and permeability between 0.025-0.61 cm/sec. It consists of Portland cement as binder and they are produced by using super plasticizer and cohesive chemical additives in order to improve some performances (Gesöğlü et al., 2014; Boğa, 2015; Guan et al., 2021). While the aggregate volume is between 60-70% in conventional concrete, this ratio is in the range of 50-65% in permeable concrete. In addition, the aggregate/binder ratio is an important

parameter in permeable concrete, and the aggregate/cement ratio varies between 4:1 and 6:1 (Akkaya and Çağatay, 2016).

In the production of permeable concrete, limestone-quartzite-dolomite are widely used as the main element with the coarse aggregate. Dolomite is accepted as the most suitable aggregate for the production of permeable cement concrete due to its high compressive strength and corrosion resistance (Lian and Zhuge, 2010; Guan et al., 2021).

As it is used in conventional concrete, drinkable water can be used as mixing water in permeable concrete. Moreover, the retarders and hydration regulators are also widely used as additives. It is seen that the air-entraining admixture improves the freeze-thaw resistance of permeable concrete and water-reducing additives are frequently used (Akkaya and Çağatay, 2016).

Alkan et al. (2021) measured the permeability performance of the permeable concrete application with a simple and reliable infiltration test on the bicycle and pedestrian path. They measured the infiltration rate of permeable concrete surfaces by using the standard test method for permeable concrete infiltration rate. The test was applied at six different points. According to the tests carried out in the field, the infiltration rates of the permeable concrete surfaces in the research area vary between 524-3194 mm/h.

Akkaya and Çağatay (2016) investigated the gradation, which is one of the important parameters affecting the physical properties of permeable concretes feeding underground water resources. As a result of the study, they found that the addition of sand can significantly increase the compressive strength.

The test results showed that the presence of blast furnace slag and basaltic pumice in concrete has positive effects on abrasion resistance and water impermeability. The most important feature considered in the

production of durable concrete is the gap structure and ratio of the concrete. The permeability of the hardened concrete affects the resistance of the concrete to the events that it may encounter during its service life. The progress of the fluids in the concrete is due to the cavities of the concrete and the harmful substances are transported into the concrete in this way. So, the concrete used in the construction of the structures such as dams must be impermeable (Binici et al., 2010).

There is a direct relationship between the abrasion and permeability of concretes and the amount of blast furnace slag and granulated basaltic pumice additives. While the samples with blast furnace slag and granulated basaltic pumice have the lowest permeability values, only the samples with granulated basaltic pumice have the lowest abrasion resistance and the highest permeability. It is known that granulated basaltic pumice and blast furnace slag can be used as fine aggregate in the concrete production of structures where abrasion and permeability are important. When additives in particular ratios are used as fine aggregate, the effect level of harmful chemical and physical destructions can be reduced, since more impermeable concrete will be produced. With basaltic pumice, which is abundant in Turkey, concrete with higher durability can be produced. More abrasion of pumice-added samples is related to its highly permeable structure. In addition, the less permeability of both blast furnace slag and pumice added samples is due to the gap-free structure of the slag (Binici et al., 2010).

## **CONCLUSION**

Permeable concrete applications make significant contributions to environmental and economic sustainability (Anonymous, 2022b). These are listed below;

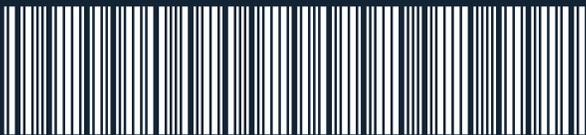
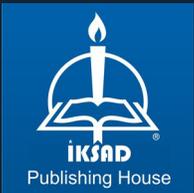
- Renewal of groundwater by infiltration of rain-flood water and runoff water,
- By acting as a filter against surface contaminants, it allows water to mix with groundwater in a clean way. Thus, it helps to control the surface contaminants.
- It helps in providing erosion control with the permeability of surface waters.
- Permeable concrete reflects the incoming sunlight because it is light-coloured and it contributes to reducing the heat island effect in cities by transmitting heat-water with its granular structure.
- Permeable concrete reduces the risk of glaciation by accelerating snow melt.
- The water can be stored with permeable concrete. Thus, it can create an important alternative for irrigation systems and it can reduce the use of drinking water.
- Permeable concrete, thanks to its superior durability, can reach a life of 30-40 years with the right workmanship and design.
- Permeable concrete can be used in landscape design by feeding tree roots thanks to its air and water permeability.
- Permeable concrete coatings can be made on flat areas. Because there is no need for slope calculations for remove the rainwater like traditional pavements. Especially, this ensures a significant contribution in sports fields.
- Permeable concrete, thanks to its light-colour, has a reflective feature and reduces the lighting cost of the area.
- Permeable concrete coatings are recognized by green building certification systems, which has started to be given importance in Turkey.

- Since permeable concrete coatings can also be used with local materials in the region, economic and environmental impacts are low.
- Permeable concrete applications are generally used in pavements and roads, car parking areas, slope stabilization, greenhouses, water entertainment centers and zoos, hydraulic structures, landscaping and sports facilities (Anonymous, 2022b).

## REFERENCES

- Akkaya, A., Çağatay, İ.H. (2016). An Experimental Study on the Compressive Strength of Permeable Concretes. *Çukurova University Journal of the Faculty of Engineering and Architecture*, 31 (2): 209-216 pp.
- Aksay, C.S, Ketenoğlu, O., Kurt, L. (2005). Global warming and climate change. Selçuk University Faculty of Arts and Sciences, *Journal of Science*. 1(25):29-42.
- Alkan, M.Ö., Kaçmaz, G., Hepcan, Ş., Hepcan, Ç.C. (2021). Measuring the infiltration performance of permeable concrete: Peynircioğlu Stream Park, Mavişehir, İzmir. *ADÜ Journal of Agriculture*, 18(2):225-231— doi: 10.25308/aduziraat.935030.
- Anonymous, (2022a). Permeable concrete, porous concrete and its properties. <https://insapedia.com/gecirimli-beton-poroz-beton-ve-ozellikleri/>. Access date: 12.08.2022.
- Anonymous, (2022b). Application guide of the permeable concrete. [gecirimli beton uygulama kılavuzu\\_147.pdf](https://www.thbb.org/gecirimli_beton_uygulama_kilavuzu_147.pdf) (thbb.org). Access date: 12.08.2022.
- Bayraç, H.N., Doğan, H. (2016). The effects of climate change on the agricultural sector in Turkey. *Eskişehir Osmangazi University, Journal of economics and administrative sciences faculty*, 11(1), 23- 48.
- Binici H., Görür, E.B., Durgun M.Y. (2010). Mechanical Abrasion and Permeability of Ground Basalt Furnace Slag and Ground Basaltic Pumice Concretes. *Electronic Journal of Construction Technologies*, 6(1): 1-10.
- Boğa, R.A. (2015). The effect of basalt aggregate use on mechanical properties in the production of permeable concrete. *Electronic Journal of Construction Technologies*, (11): 1,1-10.
- Canlı, K. (2010). Effect of global warming on forest ecosystem. *Journal of Mehmet Akif Ersoy University Science Institute*, 2: 86-96.
- Çabuk, S.Ö. (2011). Evaluation of the role of economic instruments in combating the increase in greenhouse gas emissions that cause global warming: the example of the energy sector. PhD Thesis, Ankara University Social Sciences Institute, 368 pp, Ankara.
- Gesoğlu, M., Güneyisi, E., Khoshnaw, G., İpek, S. (2014). Investigating properties of pervious concretes containing waste tire rubbers. *Construction and Building Materials*. (63): 206–213.
- Gönül, İ. A., Çelebi, G. (2003). Methods of prevention of humidity caused by the floor in buildings. *J. Fac. Eng. Arch. Gazi Univ*. 18 (4): 109-122.
- Guan, X., Wang, J., Xiao, F. (2021). Sponge city strategy and application of pavement materials in sponge City. *Journal of Cleaner Production*.
- Küçükklavuz, E. (2009). The effects of global warming on water resources: The case of Turkey. MSc Thesis, Harran University Department of Economics, 134 pp. Şanlıurfa.
- Lian, C., Zhuge, Y. (2010). Optimum mix. design of enhanced permeable concrete: an experimental investigation. *Construct. Build. Mater*. 24 (12): 2664-2671.
- Ölgen, K.M. (2010). Spatial distribution of annual and seasonal precipitation variability in Turkey. *Journal of Ege Geography*. (19)1: 85-95, İzmir.

- Tatar, V. (2018). Effects of greenhouse gas emissions on climate change: Current situation analysis in Turkey. *Journal of Social And Humanities Sciences Research*. (5) 30: 3993-3999.
- Tıraşçı, S, Erdoğan,Ü. (2021). The Effect of Global Warming on Agriculture. *Journal of Agriculture, Food, Environmental and Livestock Sciences*. 2(1): 16-33.
- Türkeş, M. (1990). Arid Regions and Important Dry Years in Turkey. PhD Thesis, Istanbul University Institute of Marine Sciences and Geography, 195 pp.
- Uysal, Y. (2022). The role of local governments in combating climate change and global warming: Findings and recommendations. *Journal of Kesit Academy*. 8 (30): 324-354.



**ISBN: 978-625-8213-37-9**