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PREFACE

Particularly in wealthy nations, the agriculture sector is facing disruptive technological advancements. Although adoption is increasing slowly in developing nations, it is however increasing. Agriculture is more profitable for farmers when it is practised properly. A proactive approach to farming is made possible by having access to site-specific data, weather forecasts, and yield predictions. A combination of technologies enables simple, accurate, and quick farm operations. With the introduction of Internet-of-Things (IoT) devices, sensors, and automated systems, modern agriculture has made it possible for even farmers to work from home. Emerging trends in agriculture indicate a shift towards intelligent farming and the effective use of time and resources while minimising crop losses. An emerging idea called "smart farming" applies artificial intelligence (AI), computer vision, and Internet of Things (IoT) to farming. By replacing up manual agricultural tasks like fruit picking, weeding, and watering, robots and drones are increasing farm automation. A high-resolution and site-specific view of the field is provided by imagery from drones and satellites combined with the Global Positioning System (GPS). Other advances in plant research and agriculture include regenerative agriculture, bee vectoring technologies, IoT soil sensors, laser scarecrows, robotic harvesting, robotic weeders, farm automation, AI-powered crop planning, chemical sensors, minichromosome technology, slowly drip to plants, plant disease detection through apps, farm management softwares, precision agriculture, resource use efficiency techniques, indoor vertical farming, hydroponics, aeroponics, fogponics, automated dairy installations; drones for livestock tracking, geofencing, grazing monitoring, livestock monitoring, crop cultivation and aerial pollination; developing seeds and tissues with increased protein content; compost additive that accelerates matter breakdown; battery-powered devices used for the measurement of pressure, flow, level, water quality, and temperature and many more.

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CHAPTER 1

RESEARCH OF THE POTENTIALS OF PREFERENCE OF 'CORNELIAN CHERRY [*CORNUS MAS* LINNAEUS (CORNALES: CORNACEAE)]' FRUIT AS AN ORGANIC PRODUCT BY CONSUMERS IN TERMS OF SUSTAINABLE CONSUMPTION

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Introduction

Chemical control is one of the options commonly used in the fight against diseases, pests and weeds that occur within the scope of agricultural production in today.

However, in this control system, pesticides used in the cultivation phase of the food that comes to our table, unfortunately, cause irreversible diseases and severe damage to all living things and ecosystems, especially humans.

For this reason, the application of environmentally friendly approaches in the process from the production of the agricultural products to the harvest and marketing and minimizing the negative effects of these approaches on natural ecosystems as much as possible constitute an important step in the concept of safe food for living things (Anonymous, 2009; Godfray et al., 2010; Foley et al., 2011; Gregory and George, 2011).

In keeping with this purpose, in the world, most agricultural producers in more developed and developing countries are turning to an agricultural production system that is 'sustainable and environmentally friendly' in order to be able to grow completely organic products. Within the framework of this ecological agricultural production system, the concept of 'sustainable consumption' has emerged now.

Sustainable production and sustainable consumption factors, which aim at the principle of 'sustainability', are the building blocks and inseparable parts of the economic development and sustainability of countries, as well as fulfilling their functionality by not harming the ecosystem (Ponikowska, 2016; Wang et al., 2019; Jerzak and Śmiglak-Krajewska, 2020).

Sustainable consumption is a concept associated with consumers' increasing interest and orientation towards organic products, as well as their willingness to purchase these products (Glomsrød and Wei, 2018; Dong et al., 2020; Han, 2020; Shabbir et al., 2020; Boca, 2021; Glavič, 2021; Weber et al., 2021; Wojciechowska-Solis and Barska, 2021). Well, which are the organic products in the scope of sustainable consumption in general, and most importantly, according to which characteristics/criteria do conscious consumers choose these products?

Today, consumers of both sexes, regardless of women/men, commonly prefer organic products such as eggs, fresh fruits and vegetables, honey, milk and cereal products. The tendency of consumers to buy these products is that there are no chemical/toxic harmful additives in the content of organic products and during their production, and that these substances are never used, the products have no negative effects on living health, on the contrary, important factors such as the presence of live health-improving properties and a greater interest in products with a pleasant smell / taste are effective (Fischer and Garnett, 2016; Baudry et al., 2017; Anisimova et al., 2019; Ditlevsen et al., 2019; Kushwah et al., 2019; Shin and Mattila, 2019; Molinillo et al., 2020).

As a fresh fruit, within the scope of sustainable consumption, one of the organic products known to have beneficial effects on health by consumers and widely preferred is the ‘cornelian cherry’ fruit. Cornelian cherry plant, known in Latin as *Cornus mas* Linnaeus (Cornales: Cornaceae), is a group of hard-core fruits, the vast majority of which consist of trees and shrubs, mainly in Central and Eastern Europe that grows and can be grown naturally in our country as well. Dogwood, whose tree height can reach heights of 7-8 m on average, generally prefers mild climatic conditions and soil structures with drainage (Klimenko, 2004; Yılmaz et al., 2009; Rop et al., 2010; Ercişli et al., 2011; Ersoy et al., 2011; Da Ronch et al., 2016; Czerwinska and Melzig, 2018; Szczepaniak et al., 2019).

Cornelian cherry, which grows naturally in our country, mostly in the coastal parts of the Black Sea Region, is a very useful fruit with many colors such as yellow, red, pink and purple. Cornelian cherry fruits, which are delicious from sour to sweet, are very rich in most phytochemicals such as flavonoids, antioxidants, anthocyanins, phenolic acids, tannins, sugars, organic acids and vitamins (Polatoğlu and Beşe, 2017).

Due to the fact that it has various benefits on many important immune-disrupting diseases, especially cancer, in people who consume its fruits, the interest of consumers of organic products to cornelian cherries continues to increase every day (Kazimierski et al., 2019; Pisoschi et al., 2021).

An Examination of Some Important Factors Influencing Consumers' Tendency to Prefer Cornelian Cherry Fruit

Some important parameters can be effective in the trends of preference and purchase of cornelian cherry fruits by consumers. These parameters are factors such as the absence of harmful additives in the product content of cornelian cherry fruit and the freshness of the fruit, the absence of any harmful chemicals such as pesticides or fertilizers on fruits and trees during the production of cornelian cherry fruit, the positive and healing effects of cornelian cherry fruits on human health, the pleasant smell / taste of cornelian cherry fruits.

The Disuse of Harmful Additives in the Product Content of Cornelian Cherry Fruit and the Freshness of the Fruit

When choosing any organic product by conscious consumers within the scope of sustainable consumption, the absence of harmful additives in the product and the freshness of the products are definitely taken into account.

When selecting any product, the criterion of whether that product has additives or not is a situation that is particularly important for consumers. So much so that when consumers examine a product they may want to buy, if they look carefully at its shape and detect products with a larger shape than normal, they may describe these products as "hormonal and additive products" as a common belief and give up on purchasing such products.

When the general condition of cornelian cherry fruit is examined from the point of view of such a criterion, which consumers observe with importance, it is concluded that the fruit can easily pass this criterion. Because the overall appearance sizes of cornelian cherry fruits are close to each other, and since there can be no cornelian cherry fruit larger than normal cornelian cherry fruit sizes, it is thought that this positive criterion can be a factor that can relieve consumers quite a lot.

On the other hand, when choosing a product by consumers, whether that product is fresh or not is another criterion that can affect their choice. As a matter of fact, the vast majority of consumers show interest in fresh products, and they move away from shapely degraded and softened products.

Since cornelian cherry fruits are in the category of fresh products, they have successfully passed this criterion, which is requested by consumers. Because cornelian cherry fruits are a group of fruits that deteriorate quickly shortly after being harvested from trees, that is, their shelf life is short. Due to these features, cornelian cherry fruits are immediately released to the market by the producers and offered to the consumers shortly after they are harvested.

Cornelian cherry fruits are among the fruits that can always maintain their freshness because they are quickly presented to the consumer. For this reason, it is thought that cornelian cherry fruits are in a very advantageous position among organic products preferred by consumers in terms of product freshness.

The Disuse Any Harmful Chemicals on Fruits and Trees During the Production of Cornelian Cherry Fruit

Within the scope of sustainable consumption, when choosing organic products by careful consumers, particular attention is paid to the criterion that no harmful chemicals have been used on the product. In this regard, most consumers prefer slightly more stained-speckled products instead of completely spotless-speckless-rust shiny products. In fact, as a common belief among consumers, "wormy fruits and vegetables without chemical pesticides" are considered healthy products.

In fact, it is a relatively proven fact that such a belief held by consumers can be true. Because, some agricultural producers can use agricultural medicines in the agricultural products they grow, in the face of each disease or pest they detect, without taking into account the population density of the disease or pest and the level of economic damage, unfortunately quite unconsciously and at high rates.

The fact that manufacturers use chemical drugs against diseases and pests that have not caused economic damage to their products is not useful, on the contrary, it is quite harmful. Because the intensive and continuous use of agricultural medicines on products does not kill the diseases and pests detected in those products, but on the contrary increases their resistance to chemical drugs every day. As a matter of fact, diseases and pests are now able to recognize agricultural medicines that are intensively used by manufacturers and become immune to them.

In addition, in addition to the fact that the diseases and pests detected in the products gain resistance to the agricultural medicines used, chemical drugs used indiscriminately by manufacturers can cause serious negative effects on living and environmental health and and by disrupting the natural balance and relations that exist naturally between consumers and producers, it can turn them upside down (Yıldırım, 2008; Hernández et al., 2013; Bernardes et al., 2015; Kim et al., 2017; Mahajan et al., 2018; Plaza et al., 2019).

For these reasons, the criterion of not using any chemical/drug on the products by agricultural producers is very important for consumers who protect themselves and the nature they live in.

When this criterion is considered in terms of the production processes of cornelian cherry fruits, the fruits are considered to be lucky according to this criterion. Because, in the production process of cornelian cherry fruits, there is no general need for the use of agrochemicals on the fruits by the producers (Daughtrey and Hagan, 2001).

Cornelian cherry fruits are resistant to many diseases, thanks to some phytochemical contents they contain, and they do not attract harmful insects, thanks to substances such as p-coumaric acid and quercetin in their content. In other words, cornelian cherry fruits are one of the fruit groups that are very lucky in terms of not seeing diseases and pests. Thanks to these features, agricultural producers are both economically relieved and protect the health of life and the environment, since they do not use any chemical drugs on fruits (Witte et al., 2002; Krzyściak et al., 2011).

Positive and Healing Effects of Cornelian Cherry Fruits on Human Health

Within the scope of sustainable consumption, another factor that is especially paid attention to by consumers is the fact that the agricultural products purchased can be good for their own health when consumed as food, and the positive and healing effects of the products can be found on their health.

In recent years, there has been a significant increase in the trend of paying attention to health, especially among conscious consumers. It is known that especially when organic products are consumed, it improves the health of people and increases immunity.

The more immunity can be strengthened in humans, the more defense mechanisms are created against the diseases that may occur and the chance of defeating the diseases increases. Especially the Covid-19 disease factor, which emerged all over the world in 2019, is perhaps the most striking example of this.

People have become aware that if their immune system is weak / rather weak, it becomes even more difficult to defeat this virus disease. They reached this awareness, unfortunately, by seeing that people with low immunity in their environment are heavily affected by this disease or by witnessing this on various social media tools.

With this awareness, people have tended more towards the search for additive-free-organic products in order to strengthen their immunity better, and by consuming these products, they have begun to attach great importance to their health.

Cornelian cherry fruits are a special group of fruits that have many beneficial properties on human health thanks to their useful phytochemical compound content. Some important phytochemicals commonly detected in cornelian cherry fruit are iridoids, anthocyanins, phenolic acids, flavonoids, ascorbic acids, carotenoids, organic acids, tannins, terpenoids, vitamins, carbohydrates, fatty acids and aliphatic hydrocarbons (Tural and Koca, 2008; Bijelić et al., 2011; Deng et al., 2013; Kucharska et al., 2015; Szumny et al., 2015; Hosseinpour-Jaghdani et al., 2017; Tiptiri-Kourpeti et al., 2019).

It is known that many serious ailments, especially cancers, can be cured in people who consume cornelian cherry fruit, thanks to their phytochemical compound content (Dinda et al., 2016; Marjanen et al., 2016; Świątowy and Szalonka, 2018; Machnik and Lubowiecki-Vikuk, 2020; Szczepaniak et al., 2021; Nawrot et al., 2022).

Cornelian cherry fruits have “antidiabetic” features. In people who consume fruits, reducing blood sugar by reducing high sugar levels is a very positive feature, especially for diabetics (Narimani-Rad et al., 2013; Asgary et al., 2014).

Cornelian cherry fruits are one of the fruits with strong “antioxidant activity”. Antioxidants are an important substance that strengthens the immune system of people and has health-healing features. Antioxidants fulfill these functions by repairing or preventing DNA damage that may occur in the cells of living things. Cornelian cherry fruits are fruits with quite good antioxidant capacity thanks to the important components they contain, such as phenolic acids, ascorbic acids and carotenoids (Hosu et al., 2016; Moldovan et al., 2016).

Cornelian cherry fruits are a group with a developed "anti-inflammatory activity". Anti-inflammatory activity is a common name given to substances and treatments that eliminate inflammatory and infection-related diseases that occur in humans. Cornelian cherry fruits are fruits that have a strong anti-inflammatory activity, especially through the various phenolic acids they contain (Choi et al., 2011; Desai et al., 2018).

Cornelian cherry fruits are known to improve kidney health in humans and protect them against hepatitis-related diseases (Es Haghi et al., 2014; Saei et al., 2016).

It is also known that cornelian cherry fruits, thanks to the phytochemical compounds they contain, are good for cardiovascular and digestive system diseases in living creatures that consume them (Soltani et al., 2015; Kaya and Koca, 2021).

The presence of positive and healing effects of cornelian cherry fruits on human health through the phytochemical compounds they contain seems to be a strong potential for consumers within the scope of sustainable consumption to prefer this fruit as an organic product.

Pleasant Smell/Taste of Cornelian Cherry Fruits

Consumers within the scope of sustainable consumption, when they tend to buy any organic product, they pay attention to the general appearance of the products they prefer, as well as they want them to have an attractive pleasant smell and taste. Cornelian cherry is a delicious fruit that has a taste scale from sour to sweet tastes (David and Moldovan, 2015). It is known that the pleasant taste property of cornelian cherry fruit is caused by organic acids such as malic acid and quinic acid in its content (Szczepaniak et al., 2021).

In addition to their pleasant smell and taste, cornelian cherries can also attract consumers with their fruits in many colors such as yellow, red, pink and purple. It is thought that cornelian cherry fruits, which have positive color/smell/taste characteristics to appeal to most consumers, have a fairly good potential in consumers' organic product selection preferences.

Conclusions and Recommendations

Nowadays, the realization of sustainable economic development in most countries is very closely related to the concepts of sustainable production and sustainable consumption. In order for a country to maintain its level of economic prosperity, sustainable consumption is very important as well as sustainable production, and the trends towards this form of consumption are increasing every day.

If the definition of sustainable consumption is to be made briefly, it is a consumption concept in which conscious consumers show an orientation to organic products compared to normal products and tend to buy such products. Within the scope of this consumption, a number of important parameters are effective for consumers to choose organic products.

Cornelian cherry fruits are one of the fruits that have a strong potential to be evaluated by consumers as an organic product within the scope of sustainable consumption. Some important parameters can be effective in consumers' preference processes for cornelian cherry fruits and their tendency to buy the fruit.

These parameters are factors such as the absence of harmful additives in the product content of cornelian cherry fruit and the freshness of the fruit, the absence of any harmful chemicals on fruits and trees during the production of cornelian cherry fruit, the positive and healing effects of cornelian cherry fruits on human health, the pleasant smell / taste of cornelian cherry fruits.

Within the scope of this research, some of the important factors mentioned above that may be effective in the potential of choosing cornelian cherry fruit as an organic product by consumers within the framework of sustainable consumption have been examined and all parameters have been discussed extensively. As a result, taking into account all the parameters studied in detail on the subject, it was concluded that cornelian cherry fruits have the potential to be preferred by consumers as an organic product within the sustainable consumption area.

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CHAPTER 2

PESTS IN LEGUMES AND THEIR EFFECTS

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1. INTRODUCTION

People meet their nutritional needs from animal and plant sources. In addition, 70% of the world's protein needs are provided from plant sources. From this point of view, legumes containing vegetable protein are very suitable in terms of superior nutrition, phosphorus, iron, and vitamins (Azkan, 1989). Legumes constitute 1.9% of the total agricultural land of our country. Legumes are sought-after crops in crop rotation systems due to their high protein content, feeding, and nitrogen-fixing properties. It is known that legumes were used as food in ancient times by the Mediterranean, Mesopotamians, Egyptians, Hungarians, Trojans, and the British.

Plants belonging to the legume family can be grown in all climatic conditions of the world except the polar regions. Among the 12 thousand species known as annual and perennial, only 200 species are cultivated. Among these species, those known as legumes are beans (*Phaseolus vulgaris* L.), chickpeas (*Cicer arietinum* L.), lentils (*Lens culinaris* Medik.), broad beans (*Vicia faba* L.), cowpea (*Vigna sinensis* L.) and peas (*Pisum sativum* L.) (Gülümser, 2016).

Legumes have been a source of protein for more than 2 billion people in the world. It is low in fat, high in carbohydrates, and nutritious. In the world, 22% of vegetable proteins and 7% of carbohydrates in human nutrition, 38% of proteins, and 5% of carbohydrates in animal nutrition are met from legumes (Gülümser, 2016).

The production of legumes should be expanded in large areas. Yield losses will be reduced by the widespread use of machine harvesting. In addition, it is possible to make a more profitable production by reducing the cost. The lands should be leveled in order to expand the machine harvesting. In addition, the use of chickpea varieties with vertical or semi-vertical characteristics is of particular importance. Fertilization, irrigation, weeds, disease, and pest control techniques are not applied properly by our producers in legume cultivation (Ton et al., 2014). In addition, some of our producers do not use too much nitrogen fertilizer. Rhizobium bacteria of legumes other than dry beans and cowpea are naturally found in the soil and can generally fix enough nitrogen without the need for bacterial inoculation. Therefore, 2-4 kg da^{-1} of pure nitrogen applied to the soil before planting is sufficient. Nitrogen fertilizer, which is given more than necessary, adversely affects the function of nitrogen-fixing Rhizobium bacteria in the soil. At the same time, excess nitrogen causes an increase in vegetative growth in legumes, as well as a decrease in seed yield. Some of our producers, on the other hand, achieve low yields because they do not fertilize. Bruchus causes great yield losses in legumes, especially in lentils. Effective chemical control should be made against this pest during the flowering period of the plant. In addition, effective chemical control should be carried out with pests such as sitonia, mulberry wisteria, leaf gallery fly, and parasitic plants such as broomrape.

Tall varieties tend to lie in the Southeast Anatolian Region. Plants that are oversized in rainy years are adversely affected by stem rot caused by sclerotinia. In this case, lentil production should be suspended. For this reason, importance should be given to the production of lentil varieties that do not show short stature (Özdemir, 2002). In edible legumes, it is necessary to develop high-quality and high-yielding varieties that are resistant to diseases, and suitable for machine harvesting. Yield and quality to be obtained after sowing can only be obtained from healthy plants. For this reason, it is necessary to follow the disease and pest damage and make the necessary fight. For this reason, it is necessary to know leguminous plant pests and diseases very well and to apply the necessary control methods. Anthracnose and rust disease are among the most common diseases in bean cultivation. It is necessary to combat these diseases in a timely manner. Aphids are among the important pests in bean cultivation and they can cause great damage.

The Chickpea plant is exposed to many diseases and pests during its development period. *Anthracnose, Rhizoctonia root rot, Pythium rot, Fusarium wilt, white mold, bacterial blight,* and some viral diseases are among the diseases that can be seen in chickpeas. However, the most common and most damaging disease in chickpea agriculture is anthracnose.

Lentils are among the important plants cultivated in our country, especially in the Southeastern Anatolia Region. With the increase in the cultivation areas of lentils, plant protection problems have also increased. Among the important pests of lentils, there are lentil seed beetle, apion, root cochlea, lentil weevil, and lentil green worm.

Anthracnose, rust disease, root collar rot, and broad bean mosaic virus are the leading diseases that cause damage to pods. Aphids and buruchus are the leading pests of broad bean. A timely and regular method of struggle should be maintained against these pests.

2. HARMFUL TOOLS AND FUNCTIONS IN LEGUMES

2.1. Acarina

2.1.1. Tetranychidae (Tetranychus urticae (Syn. T.cinnabarinus)) C. L. Koch, 1836)

Red spinners live among silky webs on the underside of the leaves of their host plants. Most of the time, it is possible to see it together in the adult, larva, nymph, and egg stages. Females have a body length of 0.4 mm and a width of 0.3 mm. Abdomen red and cephalothorax yellow. The females turn bright and dark red after feeding for a while. Their legs and bodies with red tips are hairy. They have four-part black spots on the back of their body. Females are larger than males and their abdomens are oval. The male has a body length of 0.3 mm and a width of 0.2 mm, and the color of the tips of the legs is yellow. Males are more active than females (Anonymous, 2022a).

Type and Spread of Damage

They feed by sucking the leaf sap of the plant they live on. The sucked leaf turns yellow. The amount of chlorophyll in the plant decreases, assimilation regresses and the leaves curl and fall. The quality and quantity of the product taken from the damaged plants also decrease. It appears as if the plants are covered with a mesh layer and it dries the plant. They are pests seen in almost all parts of our country, including the Aegean, Mediterranean, Thrace, and Central Anatolia Regions (Anonymous, 2022a).

Hosts

It is a polyphagous pest. It is harmful to pepper, tomato, melon, watermelon, cucumber, zucchini, eggplant, bean, pea, cowpea, cotton, strawberry, peanut, sunflower, ornamental plants, and fruit trees. Many weed species are also among the hosts (Anonymous, 2022a).

Fighting Methods

Unnecessary and excessive use of chemical control causes the natural balance to deteriorate and the pest population to increase further. For this reason, methods other than chemical control should be prioritized in order to obtain successful results in the control of the pest (Anonymous, 2022a).

Cultural Measures

Plant residues contaminated with pests should be collected and removed from greenhouses or fields. The plant residues overwintered by the red spinner should be buried in the soil by tilling the soil. Weeds in the field should be struggled and nitrogen fertilizers should not be used more than necessary (Anonymous, 2022a).

Biological Control

Natural enemies, especially Phytoseids, Coccinellids, and predator thrips are very important for biological control. Necessary measures should be taken to protect these benefits and increase their effectiveness. Great importance should be given to the selection of drugs with less beneficial side effects. Biological control against red spinner mites in vegetables with Phytoseiulus persimilis can be done successfully (Anonymous, 2022a).

Chemical Control

Since they are difficult to see on the underside of the leaves, the underside of the leaves should be examined with a magnifying glass. For the first count in vegetable gardens, red spinner nymphs and adults are counted on the bottom and middle leaves of the plant in 3-5 steps during the flowering period. The number of leaves to be examined per decare is 20 for plants with small leaves such as beans and 10 for plants with large leaves such as cucumber and eggplant. Vivid red spinners on the leaves are counted and recorded with a magnifying glass. Divide the total number of live red spinners by the number of leaves counted. The number of live red spiders per leaf is found. If an average of 3 live red spiders per leaf are found in small leafy vegetables (beans) and an average of 5 live red spiders per leaf are found in large leafy vegetables (eggplant). While spraying, a hydraulic field sprayer, back sprayer (mechanical, automatic, motorized), or back atomizer is used. The application should be made so that all parts of the plant are covered with the drug (Anonymous, 2022a).

2.2. Nematoda

2.2.1. Heteroderidae (Meloidogyne ssp.)

Root-knot nematodes form large and small tumors on the root of the host plant. Males of root-knot nematodes (*Meloidogyne* spp.) are thread-shaped and females are pear or lemon-shaped. The females are 0.7-0.8 mm long and 0.4-0.5 mm wide. The males are 1.2-2.0 mm long and the larvae are 0.3-0.5 mm long. The agent spends the first larval stage in the egg and infects the plant by hatching in the second larval stage. Then, after the third and fourth larval stages, it becomes an adult. It spends the winter as eggs or larvae in plant root residues and soil. They live in light and medium soils and do not like heavy soils. When the soil temperature is above 4 ^{0}C for cold climate species and 15 ^{0}C for tropical species, the agent becomes active and can enter the plant. It has been determined that most root-knot nematode species give offspring every 3-4 weeks at 27 °C under laboratory conditions (Anonymous, 2022b).

Type and Spread of Damage

Root-knot nematodes are internal parasite (endoparasite) nematodes. They cause galls in the root system of the host plant and restrict the exchange of water and nutrients from the soil by disrupting the vascular tissues of the plant. As a result, the continuous development of the plant slows down and stops, and stunting is observed. It causes the yellowing of leaves, falling of flowers and fruits. If the infection is severe, the plant dries up completely. The size and shape of the root product varies according to the plant species and age. Yield losses caused by root-knot nematodes vary according to population density and plant variety, and its rate in vegetables is between 15-85% (Anonymous, 2022b).

Hosts

They are polyphagous pests. Root-knot nematodes have more than 2000 hosts. Many of the weeds are also suitable hosts for root-knot nematodes (Anonymous, 2022b).

Fighting Methods

Legal Struggle

Root-knot nematodes are among the pests included in the quarantine. It is forbidden to carry all kinds of plant materials, soil, and tillage tools from one place to another in the circulation of these pests both abroad and in the country. Therefore, care must be taken in internal and external quarantine practices (Anonymous, 2022b).

Cultural Measures

A rotation that includes grains for at least three years greatly reduces the populations of these pests. It is necessary to use clean plant material. In addition, it should be recommended to grow vegetable varieties that are resistant to these nematodes. In addition, the use of rapidly degradable organic fertilizers increases the saprophytic and predatory nematode population in the soil, and plant parasitic nematodes may remain under pressure (Anonymous, 2022b).

Chemical Control

Chemical control can only be done economically in areas such as greenhouses. In closed areas such as greenhouses, soil spraying with a licensed nematicide is recommended in the summer heat. In determining the spraying time of root-knot nematodes, factors such as the biological period of the nematode, the soil character, the temperature and humidity of the soil, the phytotoxicity of the nematicides to be used in the application, and whether they are systemic or not are important. Spraying against nematodes is done when the soil is tempered and the soil temperature is 15 ºC<. Spraying within the scope of the tools and machines to be used should be done with a tool or machine suitable for the drug label. Factors that can directly affect biological activity should be chosen in accordance with the purpose. In terms of spraying technique, soil moisture before spraying is very important for the effect of the drug. During the application, the soil must be tempered, and the soil operations must be completed before the application of pesticides before planting. The soil should not be cultivated after spraying. The drug label should definitely be considered in the selection of the spraying technique to be used in spraying (Anonymous, 2022b).

2.3. Orthoptera

2.3.1. Gryllotalpidae (Gryllotalpa gryllotalpa L.)

Although they live in tunnels they dig under the ground, they have the ability to fly (Ulusoy et al., 2016).

Type and Spread of Damage

Gryllotalpa gryllotalpa usually spends the winter in the soil during the adult or nymph stages. They make the nest at a depth of 5-30 cm in the soil and preferably near tuberous plants such as potatoes. The nest is the size of a chicken egg and is flat and hard inside. Adults mate in the spring in April-May. *Gryllotalpa gryllotalpa* gives offspring every 2 years in cold countries such as Russia, while it gives offspring once a year in our country (Ulusoy et al., 2016). *Gryllotalpa gryllotalpa*'s nests are not very common in cultivated lands. This species is not harmful in dry soils. They are harmful to crops grown in humus-rich moist or wet soils. In this type of soil, they open galleries and make nests. As the gallery opens, they cut off any plant roots that get in their way. They gnaw seeds and tubers and cause the death of plants, especially by damaging young seedlings. Since they are omnivores, they also feed on small insects and larvae in the soil (Ulusoy et al., 2016).

Control

Cultural Measures

In small gardens, nymphs and adults under the ground can be killed by leaving the soil under water. In addition, with good soil tillage, the pest can be kept under pressure. In addition, since it likes harmful hot soils, manure clumps are left in different parts of the garden at the end of summer, and nymphs and adults collected here in early spring are destroyed (Ulusoy et al., 2016).

Biological Control

Many bird species and poultry keep their populations under pressure by feeding on the nymphs and adults of the pest that have surfaced.

Chemical Control

According to the regions in our country, it can be fought starting from the spring months until the end of October. The most effective method is the

'Poison Bait' application. For this, 10 kg of bran is mixed slowly with 3-4 liters of water and 500 g of sugar, and then an insecticide is added. Irrigation of the field before the application increases success. As the application area increases, the poison bait to be prepared should be adjusted according to the area (Ulusoy et al., 2016).

2.4. Thysanoptera

2.4.1. Thripidae (Thrips tabaci Lindeman)

Their body shape is elongated and oval. Its color ranges from light yellow to brown, and its wings are yellowish or yellowish-gray. Antennae are 7-segmented and bristle-like with teeth length between 0.9-1 mm. Males are shorter than females. The wings of the adults are narrow and the wing edges are fringed in the form of eyelashes. Eggs are 0.3 mm long, white in color, and oval (Anonymous, 2022b).

Adults have a narrow and cylindrical body, and female individuals have cilia-shaped hairs around their wings, which is a characteristic feature. Adults are around 1.5 mm in length (mostly 1.2-1.3 mm), and their colors vary. The color of the females varies from light yellow to dark brown. Light-colored individuals may have brown spots on their abdomens. Males are smaller and pale yellow in color and are rarely seen. In the main vein of the anterior wing, there are 14-19 hairs arranged at regular intervals from the base to the tip. Antennae have 8 segments. Their eyes are surrounded by a light color. Legs yellow, middle and hind tibiae dull gray. The egg-laying tube of females is curved downwards. Eggs are 0.2 mm in size, kidney-shaped, and white in color. A female can lay up to 30-150 eggs during her life span of 18-49 days.

Eggs usually hatch within a week. The hatched larvae resemble adults, but their wings are not developed. After two larval stages, they enter the soil to pupate. Larvae are cylindrical in shape and cream-colored. The second instar larvae are golden yellow. After the larva, they become adults after the pseudo prepupa and r pseudopupa periods. They are lighter colored and less active in pupal periods. They complete the larval and pupal stages in 3-6 days each. After they become adults, they return to the plant and begin to feed. They prefer to live and feed on flower buds and flower parts of plants. Flower thrips are very active insects and give 4-6 generations a year under field conditions, 12-15 generations a year under greenhouse conditions, and 22 generations a year under laboratory conditions. They spend the winter on various plants as adults. In greenhouses, they are seen all year round (Anonymous, 2022b).

Type and Spread of Damage

The adults and their larvae suck the sap that comes out by tearing or damaging the epidermis layer of the leaves, stems, and fruits of the plants with their mouthparts. Thrips kill the cells in the feeding area and cause white silvery spots on the leaf. In addition, they also destroy the chlorophyll cells and reduce the assimilation capacity of the leaves. The leaves become brittle, and the edge is curled and turns a reddish-green color. Silvery spots appear on fruit or pods as a result of feeding and may cause fruit disorders. They also cause black spots under the leaves with their excrement. Thrips increase their damage even more in the dry season or in dry places. Thrips feed on all parts of the plant and cause damage. The silvery spots that occur as a result of feeding are more noticeable along the main vein. With the density of this species much higher, the leaves are shed. F. occidentalis especially feed on growth point, bud, and flower (Anonymous 2022b). In particular, quality problems arise in fruits formed as a result of intensive feeding on flowers. Thrips indirectly cause significant crop losses because they are virus carriers. They are also widely available in our country.

Hosts

They are polyphagous pests. Pepper, cucumber, eggplant, melon, zucchini, watermelon, tomato, bean, pea, onion, leek, garlic, potato, spinach, Jerusalem artichoke, tobacco, cotton, beet, edible vegetables, clover, ornamental plants, and many fruit trees host (Anonymous, 2022b).

Control

Cultural Measures

It is recommended to grow plants vigorously and to use resistant varieties. In addition, infected plants should be removed from the field. Appropriate tillage and weed control should be done, and clean production materials should be used. In addition, studies carried out during the pupal period can kill the pupae in the soil and contribute to the decline of the population (Anonymous, 2022b).

Biological Control

Of the natural enemies, especially Orius spp. It is very important in terms of biological control and necessary measures should be taken to protect the beneficial ones and increase their efficiency (Anonymous 2022b).

Chemical Control

In order to determine the spraying time, if the number of adults + larvae per leaf is 6-11 as a result of the counts made with a total of 25-50 leaves by entering the field, it is necessary to struggle (Anonymous, 2022b).

2.5. Hemiptera

2.5.1. Aleyrodidae (Bemisia tabaci (Gennadius, 1889))

The adult is approximately 0.55-0.87 mm long. The body is dark yellow in color. They generally appear white due to the white wax layer on the wings. The eyes are red and their antennae have 7 segments. The wings are roof-shaped over the body when at rest. Eggs are often laid scattered or in small clumps. The egg is elliptical and 0.25 mm long. The larva is oval, with three pairs of legs, and motile when it is just emerging. The pest has 3 larval stages and the next stage is called the pupal stage. A female lays up to 300 eggs. When temperatures fall below 14 °C, spawning does not occur (Anonymous, 2022b). Even at low humidity, females cannot lay eggs and adults have a high mortality rate. In the Mediterranean Region, they continue their lives during the winter season, especially in the areas where greenhouse agriculture is carried out. They give an average of 9-10 offspring per year.

Type and Spread of Damage

Adults and larvae absorb the sap of the plant, causing the plant to weaken. If the pest is intense, the development of the plant comes to a halt. The honey-sweet substance secreted by adults and larvae covers the leaves of the plant, and saprophytic fungi develop on it, causing fumagine. Closing the stomata of the harmful plant makes the plant incapable of respiration and photosynthesis, and as a result, the yield and quality of the plant decrease. At the end of this process, the plant may die. Whiteflies also play an important role in the transmission of viral diseases (Anonymous 2022b). Especially the damage they cause to vegetables and cotton is very important.

Hosts

Whiteflies are polyphagous pests. They are harmful to many plants such as chrysanthemum, gerbera, aster, solidago, gysophila, lisianthus, helianthus, poinsettia, begonia, impatiens, and diphenbahya, cotton, okra, tomato, eggplant, pepper, bean, zucchini, cabbage (Anonymous, 2022b).

Control

Cultural Measures

Since the whitefly spends the winter in weeds, weeds in the environment should be struggled with. Since the whitefly population increases in humid environments, excessive irrigation should be avoided. Ventilation should be done to reduce the humidity inside the greenhouse. Excessive nitrogen fertilizer use should be avoided, as excess nitrogen makes the plant suitable for whitefly feeding. Ventilation openings and entrances of greenhouses should be covered with 462 µm tulle (Anonymous, 2022b).

Biological Control

With the planting of the seedlings in the greenhouse, 1 yellow sticky trap per decare is hung 10-15 cm above the plants. After the adults are detected in the traps, the traps are placed opposite each other in such a way that a trap is placed on 10 m^2 (Anonymous, 2022b).

Chemical Control

Counting is made on a total of 60 leaves, one each from the lower, middle, and upper parts of at least 20 plants in an area of one decare. When 5 larvae + pupae per leaf are detected in the count, it is decided to apply pesticides. At this stage, there are no officially recommended chemical preparations. However, when a mandatory situation arises for spraying, the Technical Organizations of the Ministry of Agriculture and Forestry should be applied. A back sprayer (mechanical, automatic, motorized) or back atomizer is used in spraying. Spraying should be done early in the morning or late in the evening after the dew is lifted, and in windless weather, to cover the lower faces of the leaves (Anonymous, 2022b).

2.6. Coleoptera

2.6.1. Bruchidae (Bruchus spp.)

Although *Bruchus ervi Frölich* is the dominant species in Southeastern Anatolia and Mediterranean Regions in our country, *Bruchus lentis Frolich* is dominant in Central Anatolia, Marmara, and Aegean Regions. *B. ervi* adults are 3.5-4 mm long, dark brown, long flattened, and cylindrical. It is covered with dark brown and white hairs towards the abdomen. B. lentis adults are 3 mm long, dirty gray, with light spots on them. B. lentis eggs are white in color, flat and shiny, with an average length of 0.47 mm (Anonymous, 2022c).

Type and Spread of Damage

As the harvested product goes from the field to the barn, the pest is present in the capsule in the larval stage. Larval development period in B. ervi averages 33 days. The larva forms a gradually enlarging cavity in the grain and becomes a pupa in it. The pupal development period is 11 days on average. Adult individuals come out by making holes in the grain. They spend the winter in warehouses and grain. Both species give offspring once a year. Since the pest causes direct damage to the grains, the nutritional value of the product with holes in it decreases, the germination percentage decreases, and they lose value in the domestic and foreign markets (Anonymous, 2022c).

Natural Enemies

Triaspis thoracicus Curtis and Dinarmus laticeps species were determined as larval parasitoids of B. lentils in the Marmara and Aegean Regions (Anonymous, 2022c).

Control

Cultural Measures

In the fight against seed beetles in lentils, first of all, cultural measures should be taken into account. It is recommended to use clean seeds that are not contaminated with seed beetles. In order to reduce the adult population, the residues left in the field after harvest should be deeply buried by ploughing. In order to prevent possible contamination in the warehouse, the warehouse should be cleaned before the harvested product is put into the warehouse. No contaminated products, sacks, or materials should be placed in the warehouse (Anonymous, 2022c).

Chemical Control

The fight against seed beetle in lentils is started during the flowering period (10% flowering), and priority is given to eliminating the first adults before they leave eggs. The first adult emergence is determined by counting with a trap. Due to ecological differences, it is recommended to control the pest by applying one spray for the Aegean region and two sprayings with 10 days intervals in the other regions (Anonymous, 2022c).

2.7. Lepidoptera

2.7.1. Noctuidae (Helicoverpa armigera (Hübner))

The forewings of Chickpea green worm adults are light brown with two dark bands that run from front to back. There is a dark band on the outside of the hind wing, a light-colored spot in the middle of the band, and a dark-brown spot towards the middle of the wing. There is also a low concentration of chickpea green worms in chickpea fields. The forewings of the adults of this species are beige or greenish brown with darker brown spots on them. The hind wings are light beige with a broad black band towards the wingtips. Eggs 0.45-0.65 mm in diameter are cream-colored and spherical in shape. It has longitudinal protrusions. Eggs are laid singly or in groups (Anonymous, 2022d).

Type and Spread of Damage

The pest spends the winter in the soil as pupae at a depth of 3-8 cm. In the spring, the first butterflies begin to appear from the end of April to the beginning of May. Butterflies usually fly in the evening. Females usually lay their eggs on the middle leaves of the plant and on the lower surface of the leaflet individually or 3-20 of them together. Eggs hatch in 2-10 days. Larvae complete their development in 11-31 days depending on the temperature and become pupae in the soil. H. viriplaca gives 1 offspring per year, and H. armigera 3-5 offspring per year. Chickpea green worms are harmful by feeding on leaflets, flowers, shoots, capsules, and grains of the plant (Anonymous, 2022d).

Control

Cultural Measures

Chickpea green worms are plowed at the end of the harvest in damaged fields so that the pupae in the soil die (Anonymous, 2022d).

Biological Control

In line with the principles of integrated control, care should be taken to protect the natural enemies of the pest. For this reason, the pesticide that has the least effect on its natural enemies should be chosen for the control of the pest. Perennial shelter plants (wild rose and blackberry) and flowering plants such as mint, wild carrot, and fennel, which are food sources (game, pollen, nectar) for natural enemies at the edge of the field, should be protected (Anonymous, 2022d).

Chemical Control

In order to determine the spraying time in chickpeas, controls are started from the second half of April, taking into account the ecological characteristics of the regions. A 0.25 m^2 (50 x 50 cm) frame is used in the controls. If butterflies fly between plants and eggs are seen on leaflets, larvae control is carried out in the field. By entering the field in the direction of its diagonals, 0.25 m^2 of frames are thrown in the above-mentioned number according to the size of the field, and the plants in it are shaken and the larvae that fall to the ground are counted. When an average of 5 larvae are detected per m^2 as a result of the count, a struggle can be made (Anonymous, 2022d).

3. CONCLUSION

The control of all diseases, pests, and weeds in the field should be considered together in the integrated control programs to be applied in legume varieties. Control management should be based on the control of the main pest, disease, and weed. Population densities of the existing pests and their natural enemies (parasitoids, predators, entomopathogens) should be monitored regularly throughout the year. Diseases, pests, and weeds that have reached the threshold of economic damage should be combated. In the struggle, alternative methods to chemical control such as biological control, cultural measures, biotechnical methods, and physical and mechanical control should be used. In some fields, weed control should be done primarily in the form of tillage and manual plucking. Weed pesticides should not be used unless absolutely necessary. The control of diseases, pests, and weeds that cannot be prevented with these control methods should be done by using the most suitable pesticides. Management of pests, diseases, and weeds should be done by taking into account "Patterns of Damage", "Sampling Times", "Sampling Methods", "Economic Damage Thresholds" and "Disclosures". Thus, it is ensured that the most appropriate and economical struggle against the pest is made (Anonymous, 2022c). Considering the developments in the world, plant protection products were accepted as the easiest and most miraculous solution in agricultural control until the 1960s, and after their side effects were noticed, they began to be used very carefully (Eker, 2008). Today, the protection of human health, the environment, and biological diversity has come to the fore. It has become imperative to fight against harmful factors in plants, taking into account the agroecosystem and sustainable agricultural production. The use of chemical control, which has many negative effects, is now being investigated. As a matter of fact, many developed and developing countries have started to implement policies and strategies to reduce the use of plant-protection sunscreens since the 1980s. As a result of this, Integrated Pest Management and Integrated Product Management research and applications were started (Eker, 2008). The purpose of technical instructions is primarily to use alternative methods of chemical control. If chemical control is required, the plant protection product recommended by the Ministry should be applied at the recommended time and dose according to the technique. In addition, in terms of consumer health, attention should be paid to the time between spraying and harvest. First of all, for the health of our country's people and for our export of quality agricultural products, it should be ensured that the struggles are carried out according to the Agricultural Protection Technical Instructions. In addition, environmental pollution caused by plant protection products should be kept at a minimum level and the natural balance should be affected at the lowest level in terms of biological control (Eker, 2008).

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CHAPTER 3

DRY BEANS (*PHASEOLUS VULGARIS* L.): AN OVERVIEW

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1. Introduction

Domestication and place of origin of dry beans are both in Latin America. Globally, countries with the highest dry bean production include Myanmar, Brazil, United States, Mexico, China, Argentina and Canada. Dry beans are a rich source of protein, dietary fiber, iron, magnesium, and folate. Symbiotic nitrogen fixation of dry beans is frequently reported to be lower than other legumes. Many biotic and abiotic stresses are the major contributors for low grain yields such as drought, diseases, insects and weeds for dry beans

Dry beans (*Phaseolus vulgaris* L.), often referred to as common beans, pinto beans, snap beans, field beans, French beans, navy beans or kidney beans, are cultivated worldwide on all continents except Antarctica. Its domestication and place of origin are both in Latin America (Fageria et al., 2010). According to genetic and historical research, the domestication of *P. vulgaris* took place in two separate domestication centres in the Andes and Middle America areas, giving rise to two genetically distinct gene pools (Farid & Navabi, 2015). Molecular evidence from more recent studies on domestication and the genetic diversity of *P. vulgaris* within the two gene pools revealed that the common bean's domestication centres are in the Oaxaca valley in the Middle Americas, southern Bolivia, and northern Argentina in South America (Bitocchi et al. 2013).

Dry bean is an important grain legume crop and supply a large part of the daily protein requirement of the people of South America, the Caribbean, Africa and Asia. The majority of dry bean consumption in underdeveloped nations is as a dry seed. It is primarily eaten as frozen veggies and fresh pods in developed countries. In South America, dried beans are a staple food that everyone eats daily with rice. Seeds of dry bean have about 22% protein and is a principal source of protein for more than 500 million people in Latin America and Africa (Fageria, 2002). Dry bean is a high-value crop grown in various regions of the world. Globally, countries with the highest dry bean production include Myanmar (3.68 million tonnes), Brazil (3.05 million tonnes), United States (1.32 million tonnes), Mexico (0.94 million tonnes), China (0.92 million tonnes), Argentina (340,000 tonnes), and Canada (260,000 tonnes) (Soltani et al., 2018).

2. Food value

Protein is abundant in dry beans. Cooked dry beans provide 25% of the daily need for lysine as assessed for a 60 kg person and three times the amount of protein in one half-cup meal as compared to maize (*Zea mays* L.) (Messina, 2014). Iron and zinc are among the several minerals that are abundant in dry beans (Beebe et al., 2000). Breeding has increased the iron content of dry bean seeds to a level that is 80% greater than that of conventional types (Blair & Izquierdo, 2012). In some parts of Africa, new cultivars of high-mineral accumulating beans have already been released (Blair et al., 2010). Despite their abundant density of minerals, the bioavailability of iron and zinc in dry beans is often limited by the large amounts of dietary inhibitors, such as phytates and tannins (Petry et al., 2014).

Dry beans are a rich source of protein, dietary fiber, iron, magnesium, and folate (Winham et al. 2008). Despite their high nutritional value, beans require long cooking times to become palatable. It takes 7–11 kg of fuel wood to cook one kg of beans, in contrast to one kg of maize flour, which requires less than one kg of fuel wood to cook (Adkins et al. 2010). Decreasing the cooking times of dry beans would be especially important in areas where beans are consumed as a primary source of protein. The genetic variability for cooking time is less understood than the environmental influences on cooking time (Cichy et al., 2015). When compared to the other staple food crops of rice and maize, dry beans require relatively longer cooking times to inactivate the amylase/trypsin inhibitors, solubilize fiber, denature storage proteins, and gelatinize the granules of starch prior to consumption. As the seed ages, or is exposed to higher ambient temperatures and humidity, the cooking time increases (Shiga et al., 20014). A range of processed food products can benefit from the addition of dry bean flours as functional additives (Horax et al., 2004). Dry beans' nutrient-richness and lack of gluten offer major customers for utilising bean flour in various dietary systems (Siddiq et al., 2010).

It is possible to obtain yields of up to 93% in dry bean protein extraction by the aqueous fractionation method, resulting in concentrates with a protein content above 70%. The combination of other treatments (thermal or not), applied before or after extraction, can improve the extraction yield and the functional properties of proteins, respectively. Dry fractionation allows yields of up to 30%, which can be improved with electrostatic separation. Bean proteins have equal or superior performance to soybeans in terms of emulsifying properties, high hydrophobicity, and foaming (Ferreira et al., 2022).

3. Agronomy of dry beans in relation to biotic and abiotic stress

Despite common bean's inherent N2-fixing ability, the actual symbiotic nitrogen fixation of dry beans is often reported to be relatively lower than other legumes (Martinez-Romero, 2003). Therefore, application of N fertilizers in bean fields is recommended to achieve higher yields. However, it has been reported that the climbing and indeterminate cultivars consistently have higher nodulation and symbiotic nitrogen fixation abilities, compared with most bush-type cultivars. These greater abilities are attributed to the relatively longer period of fixation during the growth cycle in climbing type cultivars (Farid & Navabi, 2015). Selection for high levels of symbiotic nitrogen fixation, especially when performed in low-fertility soils, might result in genetic gains in common bean breeding populations. Bliss (1993) also discussed that the level of N2 fixation can vary significantly among bean genotypes and argued that reports of insufficient levels of N2 fixation in common bean were often based on observations with only a few genotypes and were conducted with unsuitable N2 fixation measurement assays. Breeding for improved symbiotic nitrogen fixation can potentially improve legume crops that are normally dependent on N fertilizers for high yields and promote the development of cropping systems that are less dependent on N chemical fertilizers. The availability of superior genotypes with higher N2-fixation ability supports the idea that symbiotic nitrogen fixation in common bean can be improved through breeding efforts (Farid & Navabi, 2015).

Many of the biotic and abiotic stresses are the major contributors for low grain yields for this crop (Fageria and Santos, 2008). In South America, drought, diseases, and insects are the major yield limiting factors for dry beans (Fageria, 2002). In the USA, under conventional dry bean production, producers typically treat seeds with insecticides and/ or fungicides and both pre- and post-emergence herbicides are used to control weeds. Furthermore, though dry bean is a legume, 45 kg N ha–1 or more is applied at planting in conventional systems as N fixation is not as efficient in dry bean as in other legumes, such as soybean (*Glycine max* L.). Demand for organically produced dry bean has been on the rise in recent years both within the United States and abroad. Producing a quality dry bean crop without the use of synthetic amendments and pesticides presents unique challenges for organic growers, especially when it comes to soil fertility, pest management (i.e., insects, diseases, and weeds) and bean cultivar selection (Hill et al., 2016).

White mold disease caused by the necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) is a major constraint to common (dry edible and snap, garden, or green) bean production worldwide. Currently, white mold is controlled with a combination of cultural practices and fungicide applications. Cultural control practices include increased plant and row spacing, orienting rows in the direction of prevailing winds, scheduling irrigations to allow plants to dry before nightfall, and avoiding excessive irrigation during blossom senescence. These cultural practices reduce disease severity but also contribute to lower yields in the absence of severe disease pressure (Soule et al., 2011). Cultivars with upright architecture, reduced branching, reduced flowering, and resistance to lodging contribute to disease avoidance by creating a more open canopy that is less conducive to white mold development (Miklas et al., 2001). Disease avoidance, however, can be overcome by moderate disease pressure and may also contribute to lower yields. Fungicides provide good disease control but are costly, and the timing and mode of application are critical for effective control (Miklas et al., 2004).

The use of partially resistant cultivars in conjunction with cultural practices and fungicide applications has been recognized as the most eff ective approach to reducing the impact of white mold disease. This strategy has been difficult to implement owing to the lack of resistance sources and diffi culties with introgressing resistance quantitative trait loci (QTL) into adapted cultivars (Miklas, 2007). Since 2001, numerous QTL conditioning partial resistance to white mold have been identified and mapped in dry and snap bean (Ender and Kelly, 2005; Kolkman and Kelly, 2003; Maxwell et al., 2007; Terpstra and Kelly, 2008). Partial resistance is mediated by these collective QTL through two distinct mechanisms: avoidance, which inhibits establishment of the infection, and physiological resistance, which impedes spread of the pathogen through the plant (Soule et al., 2011).

Dry beans are considered good hosts for both *Meloidogyne incognita* and *M. javanica* with losses that can reduce pod numbers and seed weight per plant by 65%. Several studies report the occurrence of these root-knot nematodes in *P. vulgaris* L. cultivars. Plant nematodes are hard to control, because their natural habitat is the soil (with great buffering effect) or within roots or other plant tissues. Therefore, other control strategies are being sought, with the most viable and promising one being the use of resistant cultivars. Resistance sources to *Meloidogyne* spp. have been found in dry beans; however, they are not widely used by breeding programs. Characterization of the reaction of different cultivars to the infestation by specific *Meloidogyne* species, races, or isolates is needed to identify resistant genotypes that can be effectively used in breeding programs for root-knot nematode resistance in dry beans (Ferreira et al., 2010).

Anthracnose, caused by *Colletotrichum lindemuthianum*, is a major disease of dry bean and results great yield losses (Gonçalves-Vidigal et al., 2012).

Dry bean plants have short physical stature and do not successfully compete with weeds. Weeds compete with dry beans for moisture, light, and essential nutrients, and if not adequately controlled, can substantially reduce yield (Sikkema et al. 2008; Soltani et al., 2010). In addition, weeds can reduce harvest efficiency and cause staining of the beans, which can substantially reduce dry bean quality (Radosevich et al., 2007).

Terminal and intermittent drought limits dry bean production worldwide. To address intermittent and terminal drought issues, breeders are increasing efforts to improve genetic gains of dry bean under water-limited conditions. Drought stress manifests differently due to the timing, duration, and intensity of the limiting water stress and can be amplified by other stresses such as poor soils, disease, and heat (Trapp et al., 2015). Many plant traits influence tolerance to drought stress including rooting pattern (Beebe et al., 2007), capacity to partition a greater proportion of carbohydrate to seed under stress (Rao, 2001), capacity to set pods and fill seeds under stress (Ramirez-Vallejo and Kelly, 1998; Beebe et al., 2007; Singh, 2007), reduced stomatal conductance and leaf area, and the capability to maintain turgor through osmotic adjustment. Selection for grain yield under stress provides the best opportunity for improving tolerance to abiotic stress via traditional breeding (Munoz-Perea et al., 2006). Breeding for drought is complex due to number of traits involved, quantitative inheritance, and environmental influence.

Tolerance to drought exists but is difficult to breed for because of inconsistent expression across environments (Trapp et al., 2015).

Flooding stress is a common constraint in the dry bean industry worldwide (Knodel et al., 2002). Flooding appears to drastically reduce the germination percentage (Rajashekar et al., 2014). Furthermore, significant reductions were reported in leaf area, dry root weight and chlorophyll content in response to flooding (Celik and Turhan, 2013). Because of the severe susceptibility of common bean (Phaseolus vulgaris L.) to flooding, an understanding of the genetic architecture and physiological responses of this crop will set the stage for further improvement. However, challenging phenotyping methods hinder a large-scale genetic study of flooding tolerance in common bean (Soltani et al., 2017).

4. Conclusions

Research on breeding efficient N-fixing dry beans specifically targeting organic dry bean cultivation (including studies on crop rotation, tillage, cover crops, mulching etc.) targeting high value export market expectations is a highly profitible area to be studied.

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CHAPTER 4

GENETICS AND BREEDING FOR SALINITY TOLERANCE IN CULTIVATED SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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1. Introduction

Salinity is among major threats to modern agriculture which cause inhibition and impairment of crop growth and development. Agricultural areas in arid, semi-arid, and coastal regions are particularly susceptible to the effects of climate change on soil salinity. Sunflower shows moderate but genetically variable salt tolerance. Despite being categorised as having a moderate resistance to salt, soil salinity nevertheless has a significant impact on sunflower's growth and yield. Sunflower seed yield is a slightly complex and polygenic trait impacted by morphological, physiological, and environmental factors. Due to the stationary nature of plants, a sophisticated antioxidant defence system with a grid-like structure and several enzyme components has evolved, and it is essential for plants to survive under various stressors. Breeders should employ efficient screening techniques to find salinity resistance genes. Phenotypic flexibility of sunflower may be useful for enhancing efficient salt tolerance breeding techniques.

When growing in the nature, plants are regularly subjected to a variety of extreme conditions, including heavy metal toxicity, low/high temperature, drought stress, and salt stress. A main environmental factor that have a negative impact on a plant's life and, consequently, its productivity is salinity. Developing resistant cultivars by an understanding of the physiological and biochemical mechanisms or choosing better genotypes able to function well under stress is required to increase agricultural production (Umar & Siddiqui, 2018). As demands for seed oil are expected to increase 70% by 2050 (Ramankutty et al., 2018), improvements need to be made in cultivars under production. Rapid selection on selected traits will be possible by increasing our understanding of the genetic basis of variation in the physiological systems giving tolerance to salt stress (York, 2019). Due to its high-quality oil and broad adaptability to climatic and soil conditions, sunflower (*Helianthus annuus* L.) is one of the most valuable oilseeds in the world (Khalifani et al., 2022).

2. Salinity in sunflower

Sunflower shows moderate but genetically variable salt tolerance (Temme et al., 2019a). More than 900 million hectares of land are thought to be damaged by salt at present throughout the entire planet (Khalifani et al., 2022). To address this problem in an environmentally acceptable manner, agronomic applications such as halotolerant plant growth-promoting rhizobacteria (PGPR) utilisation have ability to handle salt stress (Yasmeen et al., 2020). As an other agronomic method, applying silicon (Si) to sunflower plants also reduces the impacts of salt stress through modulating the antioxidant system, mineral nutrients, and other essential systems (Hurtado et al., 2020). Selenium also decreases salt-induced damages through stimulating the antioxidant activities, which is associated with the improvement of the K/Na ratio needed for normal photochemical functioning, resulting with better plant growth under salt stress (Habibi, 2017).

Despite being categorised as having a moderate resistance to salt, soil salinity nevertheless has a significant impact on sunflower's growth and yield (Zeng et al., 2014). Sunflower's salinity tolerance threshold is 8.4 dS/m1, and each unit of salt beyond the threshold affects sunflower seed production by 5% (Flagella et al., 2004). According to Keisham et al. (2018), plants have three primary mechanisms to cope with salinity stress: 1) osmotic stress tolerance, 2) toxic ion (Na+, Cl-) exclusion, and 3) tissue tolerance to toxic ions. Even for plants that can tolerate salt, too much salt may inhibit their growth during susceptible phases and cause irreparable physiological damage (Bajehbaj, 2010).

The susceptibility of sunflower cultivars to salt stress varies widely. Sunflower can be categorised as a salt semi-tolerant crop based on studies employing crop water-stress index (Katerji et al., 2000). In sunflower plants, salinity-induced stress causes growth abnormalities that are detectable as early as the seedling stage. It slows down cotyledon expansion in seedlings, browns the tips of the roots, and lowers hypocotyl elongation. The three main morphological aberrations in response to salt stress in young plants are delayed seed germination, decreased inflorescence, and burning (necrosis) of leaves (Gogna & Bhatla, 2019).

The vegetative parts of sunflower plants accumulate more salt and chloride ions than the reproductive sections do (Ebrahimi & Bhatla, 2011). Root cortical cells serve as a deposit site for sodium and chloride ions, determined by X-ray microanalysis. Calcium ions in the cell cannot migrate radially when sodium ions are present in high concentrations. In comparison to intracellular areas, the calcium ion content reduction in the cytoplasm is noticeably greater. To correct its deficiency in the plant, calcium ions are reallocated towards the xylem and delivered to young leaves under salt stress (Gogna & Bhatla, 2019).

Cl- accumulates mostly in the epidermal cells of roots, whereas sodium ions accumulates in the cortical cells (Ebrahimi & Bhatla, 2012). Applying CaSO4 as a source of Ca2+ externally can counteract toxic levels of Na+ accumulation. Salt tolerance in plants is facilitated by calcium ions because they tend to restrict the radial movement of sodium ions in cells. Experimental research on the plants *Arabidopsis thaliana* and rice has shown that the main fatal ion responsible for cell toxicity is Na+. However, in addition to the sodium, salinity-induced damage in soybean and sunflower is also linked to chloride ions. Chloride content in the cells of sunflower roots is higher than sodium, which is indicating differential chloride uptake by sunflower roots. In sunflower, the leaf injury in response to salt stress is caused primarily by Cl– toxicity (Ebrahimi & Bhatla, 2011).

Flagella et al., (2004) examined on how salinity affects sunflowers. Their findings demonstrated that when salt stress increased, the oil content of sunflower seeds dropped from 52% to 21%. They also claimed that at salinity levels of 3, 6, 9 and 12 dS m1, oil production reduction was 27, 52, 80, and 88%, respectively. Chen et al. (2009) investigated the impact of saline water drip irrigation at sunflower. They discovered that when the salt ratio of irrigation water increased, plant height, head diameter, yield, and seed weight declined while irrigation water usage efficiency increased. They also reported that soil salinity in soil profile could be maintained in the subsequent year after saline water drip irrigation.

Numerous studies have been conducted on the impact of Na on plant processes because of its crucial function in salinity stress (Hasegawa, 2013). Na absorption impacts the accumulation of other essential macro- and micronutrients because of its ionic size, charge, and sequestration processes. Most significantly, Na sequestration alters the K:Na equilibrium across membranes and has a significant negative impact on potassium (K) uptake (Munns et al., 2020a). However, it is also possible that other factors contribute to salinity tolerance. It is now possible to quantify a variety of elements, collectively referred to as the ionome (Salt et al., 2008), and how they relate to salinity tolerance (Temme et al., 2019b). This is made possible by decreases in the cost of Inductively Coupled Plasma Mass Spectrometry.

Nitrogen (N) may improve a plant's ability to withstand salt by changing the levels of endogenous phytohormones such as cytokinin and kinetin. Local farmers frequently use N to reduce the negative effects of salinity on sunflowers. However, insufficient levels of N limit plant growth since N also has osmotic functions in saline soils. Numerous drawbacks, including increased insect damage and problems with defoliation, source from an excess of N under non-saline environments (Zeng et al., 2016). Under low and moderate saline conditions, a moderate N rate (135 kg N ha-1) could increase sunflower photosynthetic rates, the root-to-shoot ratio, and seed production (Zeng et al. 2014). Relevant phenotypic investigations, however, have also demonstrated that the effects of N application on crop growth varied with the progression of developmental stages and were significantly different at various soil salinity levels. From the perspectives of photosynthesis and N utilisation, more explanation of the impact of coupled salt and N stressors on crop growth is required (Ma et al., 2022).

Supplemental K and chitosan improve sunflower growth and quality via controlling antioxidant metabolism and leaf turgor, as well as minimising salinity effects. Sunflower quality and growth are improved by additional K and chitosan through controlling antioxidant metabolism and leaf turgor, as well as minimising the impact of salinity (Shehzad et al., 2020).

3. Genetics and breeding for salinity tolerance in sunflower

Sunflower varieties that can tolerate salt are urgently needed, and breeding them has tremendous economic potential (Rauf et al., 2012). Thus far, crossbreeding is still a common breeding method; however, the traditional breeding methods focus on screening germplasms with desired traits, such as those with high tolerance to salinity (Li et al., 2020).

Plants display different trait values under stressful situations than they exhibit under favourable conditions. It has been increasingly popular to explicitly take into account this trait flexibility as a helpful tool for comprehending trait variation across settings. According to Laitinen and Nikoloski (2019), resilience in some traits may depend on plasticity in others. As an example, changes in the sulphur (S) content of the leaves of cultivated sunflowers are related to the compensation of growth under salt stress (Temme et al., 2019b). Examples like this demonstrate how phenotypic flexibility may be useful for enhancing salt tolerance. In addition, the discovery of discrete genomic loci underlying trait plasticity and variation in sunflower (Mangin et al., 2017) raises the possibility that trait expression and trait adjustment under stress can be separated (Temme et al., 2020).

Sunflower seed yield is a slightly complex and polygenic trait which is impacted by morphological, physiological, and environmental factors. Therefore, responding to direct selection for yield can be inefficient (Khalifani et al., 2022). Prior researches has discovered a trade-off between vigour and the impact of salt in cultivated sunflower, with the more vigorous genotypes (higher growth under ideal conditions) demonstrating a larger drop in biomass under stress (Temme et al., 2019a).

Due to the stationary nature of plants, a sophisticated antioxidant defence system with a grid-like structure and several enzyme components has evolved, which are essential for plants to survive under various stressors. Mainly, these plant enzymes are superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferases (GST), glutathione reductase (GR), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR), which work as parts of the antioxidant defence system. Together, these enzymes constitute a sophisticated system of processes that effectively reduce, buffer, and scavenge reactive oxygen species (ROS). The tolerance mechanisms in stressed plant also include non-enzymatic components, such as ascorbic acid (AA), phenolic compounds, glutathione (GSH), alkaloids, carotenoids, flavonoids, free amino acids and α-tocopherols. These enzymes not only protect various components of the cells from damages, but also play an important role in plant growth and development by modulating cellular–sub-cellular processes such as cell elongation, mitosis, senescence and cell death, and are also involved in a wide range of processes, such as cell growth/division, cell differentiation, regulation of senescence and sulphate transport, conjugation of metabolites, detoxification of xenobiotics, regulation of enzymatic activities, synthesis of proteins and nucleotides, phytochelatins and expression of stress responsive genes. Unsaturated membrane lipids, nucleic acids, enzymes, and other cellular components are all shielded from the damaging effects of free radicals by the antioxidant defence system. As a result, the scientific community has been paying close attention to antioxidant defence system in plants (Rajput et al., 2021)

In the presence of NADPH and flavine adenine dinucleotide (FAD), a byproduct of the water-soluble vitamin riboflavin, the glutathione reductase (GR) reduces oxidised glutathione to restore intracellular glutathione. Thus, during sunflower seedling growth under salt stress, NO (nitric oxide) and melatonin appear to affect glutathione reductase (GR; EC 1.6.4.2) activity and glutathione concentration (Kaur & Bhatla, 2016). Although it has also been noted in the cytosol, nucleus, peroxisomes, and mitochondria, the chloroplast in photosynthetic tissues is where GR is thought to perform the majority of its action (80%). Three GR loci were found in the *O. sativa* genome and two in the *Arabidopsis* genome, according to genome-wide association studies. It has been revealed that different plants have different number of isoform of GR (Rajput et al., 2021).

The improvement in plant performance under salinity stress is accompanied by changes in ion homeostasis, osmotic adjustment, and the activity of the glyoxalase system (glyoxalase I [Gly I] and glyoxalase II [Gly II]). These changes enhance the antioxidant defence system's protective effects. Exogenous treatment of phytoprotectants, such as the foliar administration of various chemical elicitors (such as vitamins, antioxidants, and phenolic compounds), is now being used to confer resistance to the harmful effects of salt stress on plants while enhancing ROS-scavenging capacity (Hasanuzzaman et al., 2023).

Observations suggest that S-nitrosylation and denitrosylation play a part in NO signalling, which controls a variety of enzyme activities in sunflower seedlings under salinity stress (Jain et al., 2018). S-nitrosylation, a nitric oxide-dependent post-translational modification (PTM), is crucial for plant development and environmental response. In the study of Zhai et al. (2023), sodium nitroprusside (SNP), an exogenous NO donor, reduced the tomato plant's growth inhibition caused by NaCl, especially at 100 M. The transcripts, enzyme activity, and S-nitrosylated level of GR all increased after treatment with NaCl. S-nitrosoglutathione (GSNO) was able to greatly enhance GR activity. Compared to wild-type (WT) seedlings, SlGR overexpression transgenic tobacco plants showed improved germination rate, fresh weight, and root length. The accumulation of reactive oxygen species (ROS) was lower, whereas the expression and activities of GR, superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT); the ratio of ascorbic acid/dehydroascorbic acid (AsA/DHA), reduced glutathione/oxidized glutathione (GSH/GSSG), total soluble sugar and proline contents; and the expression of stress-related genes were higher in SlGR overexpression transgenic plants in comparison to the WT plants following NaCl treatment. Following a NaCl treatment, transgenic plants accumulated more NO and S-nitrosylated levels of GR than WT plants. These findings showed that GR's S-nitrosylation significantly influenced salt tolerance through controlling the oxidative state.

Breeders should employ efficient screening techniques to find wild species that have salinity resistance genes and efficient breeding techniques to transfer these genes into cultivated sunflower genotypes. Sunflower seedlings can be selected for salt tolerance based on the emergence percentage, emergence index, shoot length, and shoot fresh weight. Salt tolerance is not associated with turgor. Proline accumulation has a greater effect on tolerance to salt. Given the relationship between callus growth, seed germination, and vigour, the former may be a more accurate indicator of salt tolerance (Škorić, 2016).

One species, the Pecos or "Puzzle sunflower," *H. paradoxus* Heiser, is adapted to very salty and alkaline soils. As a rare and endangered species, *H. paradoxus* is thought to be a hybrid that resulted from the mating of two salt-sensitive species, *H. annuus* and *H. petiolaris* (Lexer et al., 2004). If one or a few specified wild sunflower species are included in the hybridising process, there is a considerably larger chance of producing sunflower genotypes with higher levels of salt tolerance. Practically speaking, morphological, physiological, and biochemical characters that lead to salinity adaptation are important to enhance sunflower stress tolerance (Kaya & Vasilevska-Ivanova, 2021).

Miller and Seiler (2003) generated two salt-tolerant oilseed parental lines, HA 429 and HA 430, using this interspecific germplasm. When *H. paradoxus* plants were grown in graded NaCl concentrations, Welch and Rieseberg (2002) discovered that they were five times more salt tolerant than their ancestors, *H. annuus* and *H. petiolaris*. Using expressed sequence tag (EST) libraries of sunflower, salt-tolerant candidate genes that encode calcium dependent protein kinase (CDPK3) and link to a salt tolerance QTL on LG4 were found based on homology to genes with known functions and earlier QTL findings (Lexer et al., 2004). Genes involved in the transport of potassium and calcium were either constitutively under- or overexpressed in *Helianthus paradoxus*, indicating that these genes may have helped this species adapt to salinity (Edelist et al., 2009).

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CHAPTER 5

USE OF MICROORGANISMS IN AGRICULTURE

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INTRODUCTION

Although fertilizer applications are seen as a necessity in order to obtain high efficiency in agricultural production, the health of living things and the environment are adversely affected due to excessive and unconscious fertilizer application. As a result of incorrect fertilizer applications, salinization in the soil, heavy metal accumulation, nutrient imbalance, deterioration of microorganism activity, eutrophication and nitrate accumulation in water, introduction of nitrogen and sulfur containing gases into the air, greenhouse effect, etc. problems occur.

Soil is one of the most complex habitats on earth and is home to a diversity of life. Most of the macro and micro plant nutrients are obtained from the soil. Plants help the essential nutrients taken from the soil reach people. Thus, the soil organisms inhabiting the soil are extremely diverse and contribute to many ecosystem services necessary for the sustainable function of natural and managed ecosystems.

Soil biota is involved in the global cycle of organic matter, energy and nutrients and includes a wide variety of macro-organisms (micro- and macro-arthropods, earthworms and termites) and microorganisms (bacteria, fungi, algae, protozoa and some nematodes). This is an important indicator that soil biodiversity reflects the mix of living organisms in the soil. It is estimated that 25-30% of all species in the world live all or part of their lives in soils (Orgiazzi et al., 2016). Soil biota, one of the critical components of an interconnected ecosystem, is the world's most biologically diverse community, and this dynamic environment is one of the key regulators of many ecosystem functions.

Plant and animal microorganisms live in the soil, develop and die. Soil microorganisms break down plant and animal wastes in the soil, meet their energy needs, and increase soil fertility in the chemical reactions that occur as a result of the activities of microorganisms. The desired microorganism activity in the soil can be achieved by adding organic matter, drainage, liming, parasite control and inoculation of the soil with bacteria.

Soil microorganisms break down and decompose organic residues in order to meet their nutritional and energy needs, and offer nutrients to plants, animals and humans to benefit from it, beneficial soil microorganisms help the soil particles to aggregate with the gum, resin, filament and micelles they extract, and by aggregation, they affect the soil water and air, thereby protecting the soil from water and wind erosion they protect.

1. SOIL MICROORGANISMS

The microbial population of the soil consists of bacteria, actinomycetes, fungi, algae and protozoa. Bacteria outnumber the other 4 groups in the soil. However, bacteria make up less than half of the total microbial tissue in the soil, since their diameter is 0.5-1 micron, their length is 4-5 micron, and the other 4 groups are in the form of large cells or long threads. The bacterial cell is prokaryotic, the nuclear plasma is not separated from the cytoplasm. Bacterial cells are composed of peptigoglycan. Bacteria reproduce by dividing in the middle and genetic information is provided by conjugation and transduction (Sert, 1997).

Bacteria are morphologically classified into three types as rods, spheres and spirals. The most spherical ones are found in the soil. Those that use light as an energy source are called photoautotrophic, and those that obtain energy from a chemical substance are called chemoautotrophic. If CO_2 is used as the carbon source of the cell, these organisms are called lithotrophic, and those that obtain cell carbon from organic matter are called organotrophic organisms. Autochronous organisms are organisms that grow slowly in soil containing substances that are not easily oxidized (*Arthobacter, Corneobacter, Mycobacter* and *Nicordia* from actinomycetes). Zymogenous organisms are organisms (*Bacillus* and *Clastrodium*) that show excessive activity in adding fresh organic matter to the soil (Kızıloğlu, 1995; Anonymus, 2023a; Figure 1).

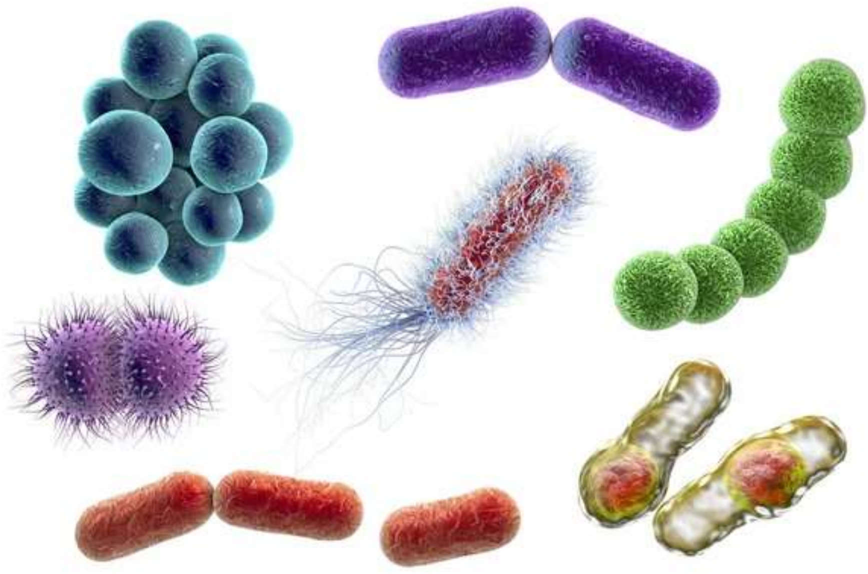

Figure 1. General bacterial appearances (Anonymus, 2023b)

Soil microorganisms are divided into 4 groups according to their sensitivity to O_2 and their activity: aerobes, anaerobes, facultative anaerobes, and microaerophiles. There are species that form spores from aerobic and anaerobic bacteria, those that form under aerobic conditions are called *Bacillus*, those that form spores under anaerobic conditions are called *Clostrodium* in the soil (Kızıloğlu, 1995).

Spores are used morphologically as a distinguishing feature in the identification of cells. The presence or absence of phlegellum and their response to gram staining are also used in the classification of bacteria. Facultative anaerobes live in both aerobic and anaerobic environments. Microerophiles require very little O_2. Microorganisms need water to feed and make new cells. Fungi are generally more resistant to high water potential than bacteria. Fungi thrive in the presence of 12% water, while bacteria thrive at 20%. The optimum humidity level for bacteria is 85%. Soil organisms are divided into three groups according to their water requirements: xerophilic, mesophilic and hygrophilous. Bacteria are hygrophilous (Anonymus, 2023a).

Humidity, temperature, weather, pH, organic and inorganic substances, processing, season and depth affect the bacterial flora. Humidity, excess water causes the separation of oxygen from the soil and the formation of anaerobic conditions. In the case of water deficiency, biochemical reactions do not occur. Moisture content of 50-75% is sufficient. Biological activity does not occur when the temperature is below 0 ^{0}C. Psychrophilic bacteria multiply at 20 °C and below, Mesophil bacteria at 20-45 °C, Thermophilic bacteria at 45-65 °C (Sert, 1997). Soil air and pH, O_2 are necessary and important. O_2 deficiency causes the formation of anaerobic environment, aerobic microorganisms need O_2, good aeration of soils is necessary for nitrifying bacteria and nitrogen fixing bacteria. Bacteria are active in soil at a pH of 6-8 (Kızıloğlu, 1995).

Figure 2. Seasonal variation in bacteria (Anonymus, 2023c)

Organic matter is the source of nutrients and energy required for the growth and activities of bacteria. Hetotrophic bacteria increase when organic matter increases. Inorganic substances are necessary for chemoautotrophic bacteria. The increase in heat, humidity and organic matter in the spring provides an increase in the bacterial flora in the soil. The high temperature and low humidity in the summer cause a decrease in the number. The decrease in temperature in autumn increases the number of bacteria slightly, as precipitation and organic matter increase. With the decrease in temperature in winter, it causes a decrease in the number and activity of bacterial flora (Figure 2).

2. USE OF BIOLOGICAL FERTILIZER IN AGRICULTURE

As a solution to the rapid industrialization and population growth experienced in the second half of the twentieth century in the world, policies aimed at obtaining high yields from the unit area with intensive use of inputs and opening new areas to agriculture were determined as targets. However, the intensive and unconscious use of pesticides/fertilizers for high yields has brought along important environmental problems such as deterioration of the physical structure of the soil, loss of soil vitality, deterioration of nutrient balance, salinization and barrenness.

Among these, it has been revealed that nitrate washed from the soil profile with excessive use of nitrogenous fertilizers pollutes the groundwater, that nitrogenous gases pass from the soil to the atmosphere as a result of the denitrification of nitrogenous fertilizers applied under certain soil conditions, and that some of these gases (for example, nitrous oxide) create a greenhouse effect and/or change the ozone layer. Similarly, the view that chemical pesticides are likely to exist or remain in the food chain and in the environment has begun to gain value. In line with these developments, biological fertilizers have become popular.

Promotes vegetative growth when applied to seed, plant surface or soil by fixing atmospheric nitrogen, increasing the availability of mineral elements from organic and inorganic sources or by producing secondary metabolites; The material consisting of living microorganisms that can colonize in the rhizosphere or enter plant tissues is called biological fertilizer (BF). With the belief that intensive agriculture necessitates the use of excessive fertilizers, excessive and unconscious application of fertilizers affects the health of living things and the environment negatively. As a result of wrong fertilizer applications, problems such as salinization in the soil, lack of nutrients, and deterioration of microorganism activity occur.

The term "rhizosphere" is derived from the Greek words "rhiza" meaning root and "sphere" meaning domain (Hartmann et al., 2008). The term was first coined by the German scientist Lorenz Hiltner in 1904 as "the region of soil adjacent to the plant root that supports a high level of bacterial activity" (Hiltner, 1904). In other words, the rhizosphere is the part of the soil adjacent to a plant's root system and affected by root exudates.

The term "rhizosphere" is derived from the Greek words "rhiza" meaning root and "sphere" meaning domain (Hartmann et al., 2008). The term was first coined by the German scientist Lorenz Hiltner in 1904 as "the region of soil adjacent to the plant root that supports a high level of bacterial activity" (Hiltner, 1904). In other words, the rhizosphere is the part of the soil adjacent to a plant's root system and affected by root exudates.

The exudates function as messengers that stimulate the interaction between roots and soil microorganisms and have a selective effect on the microorganisms present in the root zone. These compounds; It contains carbohydrates, sugar, vitamins, flavonoids, nucleotides, enzymes, hormones, organic acids, inorganic ions and gas molecules (Prasad et al., 2017). The quantitative and qualitative composition of these metabolites is determined by various environmental factors, including cultivar, plant species, plant growth stage, soil type, pH, temperature, and presence of microorganisms (Badri and Vivanco, 2009; Uren, 2000). The rhizosphere is known as a dynamic region where very important and intense interactions between plant, soil and microfauna occur because it includes plant roots and the surrounding soil. Therefore, the most active medium in the soil with a high microbial diversity is considered the "rhizosphere". There is a relationship between plant roots in the soil and microorganisms living in the plant root; The degree of influence of the plant on the microbial community increases as it approaches the root surface (Backer et al., 2018).

Different microorganisms selected from the rhizosphere are used in order to minimize fertilizer application and maximize plant growth and nutrition. Plant growth promoting rhizobacteria (PGPR) and vascular arbascular mycorrhizal fungi (VAM) are used as biological fertilizers (BF) due to their beneficial effects on plant growth. In many parts of the world, the use of PGPR as a biological fertilizer is becoming widespread in order to reduce potential pollutants, industrial fertilizer and pesticide applications (Burdman et al., 2000).

PGPRs are present in high amounts in the rhizosphere. PGPRs are free bacteria useful and important for plants and agriculture. PGPRs used as biofertilizers are low-cost, renewable and environmentally friendly fertilizers that can be applied to increase the productivity of agricultural products and reduce the sustainable application of inorganic fertilizers, facilitate the availability of nutrients and improve soil health. In order to ensure agricultural productivity without damaging the ecosystem, the application of PGPR in agriculture is very important for crop productivity, biological improvement, ecosystem functioning and biocontrol.

2.1. Biological Fertilizers Used in Agriculture and Their Benefits

Root bacteria that stimulate plant growth are generally found in genus such as *Bacillus, Lactobacillus, Paenibacillus, Arthobacter, Pseudomonas, Burkholderia, Enterobacter, Pantoae, Klebsiella, Xanthomonas, Serratia, Rhizobium, Bradyrhizobium, Azosprillium, Azotobacter*. Biological nitrogen fixation (BNF) is considered as an alternative fertilizer source for the development of sustainable agriculture, meeting changing human needs, increasing environmental quality, protecting natural resources and reducing soil erosion (Çakmakçı, 2001).

BF, together with the effect of BNF, increases plant growth by producing growth-promoting and hormonal substances, is used in the control of diseases, and provides the uptake and economic use of soil plant nutrients. Different bacterial species are isolated from the rhizosphere of field and horticultural crops, purified, their potential is revealed, and suitable microorganism mixtures are prepared as biological fertilizers.

Biological fertilizers are cheaper, do not have a toxic effect on plants, do not pollute groundwater, do not increase soil acidity. Symbiotic and nonsymbiotic nitrogen fixation, mobilization of plant nutrients, biological control of soil-borne diseases and secretion of plant growth stimulating substances are among the benefits of biological fertilizers and are the most prominent features of plant growth (Lucy et al., 2004).

3. BIOLOGICAL FERTILIZER STUDIES

Today, in many countries of the world, the effect of root bacteria, which stimulates plant development, is studied on the yield-enhancing effect of plants (Arias, 2000; Chen et al., 1996; Luz, 2000; Romerio, 2000; Wall, 2000). Studies with these bacteria started in 1979 in China, and in 1985 large-scale field applications were started. As a result of studies in China, the yield increases provided by these bacteria in some products are as follows; paddy 16.2%; wheat

11%; corn 12.5%; potato 22.5%; cotton 10.4%; sugar beet 16.9%; watermelon 15.5%; root vegetables are 20% (Chen et al., 1996). In other studies conducted in the United States on the effects of root bacteria stimulating plant growth on yield, 5-20% in barley; 57% in canola; 14-25% in peanuts; 12-24% in rice; 12-15% increase in yield has been determined in celery (Kloepper et al., 1991).

Although the first applications of PGPRs in crop production were for supporting plant growth, subsequent studies have shown that PGPRs can also be used as biological control agents in crop production. When the studies are examined, it is seen that a bacterial species can carry more than one PGPR feature. This gives PGPRs the potential of being a biological fertilizer in plant production, as well as being a biological control agent. Many of the PGPR group bacteria can also work as very good biological warfare agents. These bacteria can achieve significant success in fighting plant diseases, especially soil-borne diseases (Figure 3).

Figure 3. Bacteria Production (Anonymus, 2023d)

In a study conducted with *Bacillus polymyxa*, one of the root bacteria stimulating plant growth in Saudi Arabia, the bacteria both reduced wheat root rot caused by *Fusarium graminearum* and *Cochliobolus sativus* and increased yield by 102% (El-Meleigi & Hassan, 2000). Studies on root bacteria that stimulate plant growth have been carried out in Uruguay since the early 1960s. At first, studies were carried out on nitrogen fixation with bacteria such as *Mesorhizobium loti* and *Sinorhizobium meliloti*. The results obtained from these studies have been successfully translated into practice.

Today, all of the plants belonging to the leguminosae family are used by inoculating them with these unique rhizobiums. Studies in this country in the following years focused on the effects of these root bacteria, which stimulate plant growth, on the control of plant diseases (Arias, 2000). PGPRs are considered as indispensable elements of agricultural techniques such as Organic Agriculture and Integrated Product Management, since they can be used as biofertilizers and can be used as biopesticides in biological warfare due to their yield-enhancing properties.

4. CONCLUSION and RECOMMENDATIONS

Chemical fertilization, which is one of the most important agricultural applications, contributes to production on the one hand, and on the other hand, it can cause some negativities as a result of its wrong and unconscious application. Therefore, with the application of chemical fertilizers with a fertilization program prepared based on the analysis results under expert control, the negative effects on the environment should be reduced and economic and high yield potential should be provided.

For this purpose, soil, plant and water analyzes should be considered as the main factors in fertilizer application. Considering that the consumption of products grown in areas exposed to intense chemical fertilizers by humans and animals may cause harmful compounds in these areas to pass into living bodies, these areas should also be rehabilitated.

PGPRs should be used as biofertilizers and their use should be encouraged, since they are biopesticides in biological warfare, they are more economical and most importantly they are environmentally friendly.

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CHAPTER 6

PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AND THEIR ECO-FRIENDLY STRATEGIES FOR IMPROVING PLANT GROWTH IN SOME MEDICINAL AND AROMATIC PLANTS OF THE *LAMIACEAE* FAMILY

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INTRODUCTION

Medicinal and aromatic plants are valuable sources of herbal products globally due to their secondary metabolite components, high antioxidant capacity and many other biological activities (Şirin et al., 2021). People heve benefited from plants for nutrition, shelter, heating, healing wounds and curing disease from past to present. Many civilazition (Hittite, Egyptian, Sumerian, Assyrian and Mesopotamian) have used herbal treatment for many years. Over time, with the introduction of drugs into production, it has caused a decrease in the use of medicinal and aromatic plants. In the 20th century, the demand for natural products increased again as people discovered the side effects of synthetic drugs and realized the harm to human health caused by synthetic substances in food and beverages (Göktaş and Gıdık, 2019). Treatment with plants are used in many countries of the world under different names such as natural treatment, traditional treatment, complementary treatment (Demirezer, 2010). According to the of the World Health Organization, 80% of the population in underdeveloped countries use traditional medicines for therapeutic purposes, while this rate is around 40% in developed countries, and the rate of utilization of medicinal plants is expected to increase worlwide in the future (Acıbuca and Bostan Budak, 2018).

Medicinal and aromatic plants have been used in many fields such as food, medicine, spices and cosmetics from past to present. Plant essential oils, which are called by different names such as aromatic oil, essential oil and ethereal oil are among the important components of plant chemistry (Çelik et al., 2007). In addition to forming the structure of hormones in plant cells, they play a role in intercellular information transport and defense mechanisms of plants (Erdoğan, 2012). These oils are obtained from the leaves, flowers, seeds, bark and roots of plants, generally by using steam distillation or different extraction methods. They are mostly colorless or light yellow oily mixtures. Usually in liquid form at room temperature and easily crystallizable. Although they are defined as oils because they are insoluble in water and dissolve in organic solvents, they are different from fixed oils (Grassman et al., 2003). Monoterpenes, diterpenes, sesquiterpenes and their oxygen derivatives in hydrocarbon structure, as well as alcohols, aldehydes, esters, ketones, phenolic and oxidized components can also be found in the structures of essential oils

(İşcan, 2002). These natural products are used as antibacterial, insecticidal and antifungal due to their aromatic components (Simon et al., 1990).

Turkey is home to many medicinal and aromatic plants due to its different climate and soil structure. Some of these plants are collected from nature while others are cultivated. In order to get more yield from cultivated medicinal and aromatic plants, chemical fertilizers are used (Özmen and Ekin, 2022). Realizing the damage caused to nature and living things by the intensive and unconscious use of synthetic fertlizers, scientists have turned to nature-friendly biofertilizer applications. Approches such as enhancement of the soil rhizosphere with biological fertilizers, recycling of organic residues, more widespread use of biopesticides and the removal of pollutants in the agro-ecosystem by biological methods are essential. The cost and environmental damages of chemical fertilization has brought the research, adaptation, development and adoption of biological alternatives to the agenda and research on biological fertilizers has gained momentum (Çakmakçı, 2005).

With the rapid increase in the world population, it has become a necessity to increase the crop production. The use of the chemical inputs resulted in higher yieldes per unit area. Pesticides and chemical fertilizers used intensively and unconsciously have increased agricultural production, but have started to harm humans and the environment. Therefore, interest in sustainable and organic farming systems, which are considered environmentally friendly, has increased. One of the methods that can be an alternative to chemical applications and can be easily used in agriculture is applications that plant growth promoting rhizabacteria (Ekici et al., 2015). In the last years, the use of microorganisms has become important to reduce the need for chemical fertilizers and for sustainable agricultural activities. Bacteria that colonize the rhizosphere and plant roots and play a role in plant growth are called plant growth promoting rhizobacteria (PGPR) (Ashrafuzzaman et al., 2009). These bacteria are usually belong to the genusses *Azospirillum*, *Azotobacter, Azotobacterium, Arthrobacter, Bacillus, Burkholderia, Pseudomonas, Clostridium*, *Enterabacter* etc. (Adesemoye et al., 2018). PGPRs promote plant growth their mechanisms of action such as uptake of water and minerals, fixation nitrogen, solubilization phosphorus and other heavy metals, producing hormones (auxin, cytokinin, giberallin) and supporting root growth (Dejordjevic et al., 1987; Ferreira et al., 1987). In addition, rhizobacteria degrade pesticides (Ahemad et al., 2012), detoxify heavy metals (Ma etal., 2012; Wani et al., 2010), tolerate salinity (Mayak et al., 2014) and increase the utilization of nutrients and minerals by plants (Çakmakçı, 2009). In many parts of the world, the use of plant growth promoting rhizobacteria as biological fertilizers is becoming widespread in order to reduce the application of industrial fertilizers and pesticides, which are potential pollutants (Burdman et al., 2000).

Bacteria use some carbohydrate and protein-derived substances and carbon and nitrogenous compounds secreted in the root regions of plants called rhizosphere as energy sources (Sarma and Saika, 2014). The various substances secreted from the roots attract microorganisms and can regulate the formation of the microbial population of the soil near the root surface (Dakora et al., 2002). Bacteria, which are abundant in the rhizosphere, convert plant nutrients into nutrients useful for plants (Malua and Vassilev, 2014). PGPR applications increase germination rate, leaf area, root growth, yield, nitrogen rate, protein rate, root and stem weight, the aging of the leaves is delayed and resistance to some diseases is provided. PGPR applications can be carried out in field conditions, greenhouse and laboratory conditions (Çakmakçı, 2005).

In this review, the effects of the use of PGPR inoculants in some medicinal and aromatic plants on soil productivity, plant uptake and plant tolerance to environmental stresses will be discussed. The activities of PGPR inoculants and the results of the investigations carried out so far on related species will be presented.

2. *LAMIACEAE* FAMILY AND PGPR

Some of the medicinal and aromatic plants traded in Turkey are collected from nature (rosemary, sage, laurel, wild thyme, linden, netle, chamomile etc.) and some are cultivated (rosemary, basil, medicinal sage, fennel, mint, cumin, quinoa, lavender etc.) (Baydar, 2020). The *Lamiaceae* family, which is among the traded plant species, ranks first in the world with 224 genera and approximately 5600 species. In the flora of Turkey, there are 565 species in 45 genera belonging to this family (Duman et al., 2005). Plants belonging to this family show high biological and pharmacological activities thanks to the essential oils they contain (Özyazıcı and Kevseroğlu, 2019). The *Lamiaceae* family, one of the most important plant families, includes a wide variety of plants with biological and medicinal applications. The most well-known members of this family are mint, thyme, rosemary, basil, lemon balm, sage, lavender etc. (Bekut et al., 2017).

In the 20th century, there has been a rapid increase in the use of synthetic substances in various sectors, including agriculture, food and pharmaceutical industries. Today "green" solutions have become indispensable due to increasing environmental pollution, which is also reflected negative impacts on human health (Kralova and Jampilek, 2021). Recent studies on the production of biological preparations of plant growth promoting rhizobacteria (PGPR) have been increasing due to their positive effects on plant growth, plant systemic resistance and effectiveness in disease control (Imriz et al., 2014). In many parts of the world, the use of PGPR as a biological fertilizer is becoming widespread in order to reduce pollution caused by industrial fertilizer and pesticide applications (Burdman et al., 2000). When applied to the plant surface, seed or soil, they are living microorganisms that fix the nitrogen present in the atmosphere, increase the uptake of mineral elements from organic and inorganic sources or positively affect plant growth throught the production of secondary metabolites and can be colonized in the rhizosphere region (Çakmakçı, 2005).

Medicinal and aromatic plant species are valuable because they are consumed without much processing and the harvested product does not contain synthetic compounds. Excessive use of synthetic fertilizers has negative effects on plant and soil health. Consideing the environmental pollution and high production costs caused by excessive fertilizer use; Rhizobactera (PGPR), a plant growth promoter, can be used in sustainable agricultural production (Kutlu et al., 2019). Medicinal and aromatic plants have been known to people since ancient times. Some of them are also used as drugs in modern medicine due to the healing effects of secondary metabolites present in their structure. One way to increase secondary metabolites in medicinal and aromatic plants is the use of biofertilizer PGPR. The use of these biofertilizers is now applied to medicinal and aromatic plants a an alternative to chemicals. Microbial-based biostimulants have been very significant since the beginnig of agriculture (i.e., Rhizobium in *legumes*) and current prospects comprise their commercialization as a complement to crop nutrition. The useful effects of micoorganisms on plants depend on complicated nutrent and chemical signaling, as well as soil and climatic conditions. Plant roots emit organic acids, sugars, amino acids and phenolic that impact the composition of rhizosphere communities and lead to beneficial relationships (Ortiz-Castro et al., 2009). Several groups of mycorrhiza, bacteria and other groups of microorganismis are related to the root systems of all higher plants. Bacteria, one of the biggest groups of microorganisms, can support plant growth by attaching to the external surface of the plant, such as roots or leaves or by forming endophytic relationships on the internal surfaces of the plant (Şirin et al., 2021).

2.1. Mint *(Mentha piperita)*

Mint, an important essential oil and plant, is a general name given to *Mentha* species in the *Lamiaceae* family and is a herbaceous plant with perennial and creeping stems. *Mentha* species are commercially cultivated in many countries because of the value of their volatile network. Peppermint oil is the richest natural source of menthol, which has a wide range of applications in the pharmaceutical, food and cosmetic industries (Baydar, 2020). It is widely cultivated in China, Japan, Brazil, South Africa, Taiwan and Argentina (Akgül, 1993). Mint, which is grown in the Mediterranean climate zone, is grown in almost every region of our country. On the other hand, it is produced as a cultural plant in the Aegean, Marmara and Mediterranean regions and is a commercially produced plant (Kocabıyık and Demirtürk, 2008). There are more than 25 species of mint. Some of them have essential oil containing major components such as menthol, carvone, menthone and pulegon (Phatak and Heble, 2002). Essential oils are obtained from wild plants that grow naturally in natur or from cultivated aromatic plants. Since aromatic plants contain therapeutic components in their chemical composition, it is a well-known fact that these plants have positive effects on human health. Many biological activities of essential oils obtained from plants and their components are known. Since essential oils are complex mixtures containing different components, they also show differences in terms of their biological effects (Toroğlu and Çenet, 2006).

Plant growth promoting rhizobacteria ensure healthy growth and development of the plant. PGPRs also show positive effects in the medically important *Mentha* species. PGPRs also show positive effects in the medically important *Mentha* species. Bharti et al. (2014), reported that the negative effects on growth, oil content and physiological status of mint plants under salt stres were alleviated by PGPR. Chiappero et al. (2019), investigated the effects of plant growth promoting rhizobacteria on antioxidant, total phenolic and plant growth in *Mentha piperita* plant grown under drought stress. In the study, moderate and severe drought stress was applied with two PGPR (*Pseudomonas fluorescens* and *Bacillus amyloliquefaciens)* species. It was observed that was a decrease in plant growth, fresh weight, leaf number and leaf area with the increase in drougt stres level. However, it was reported that these negative effects of drought were alleviated in PGPR applied plants. It was reported that plants under drought stres treated with PGPR had higher total phenolic content and enzymatic activities than plants under water stres without PGPR. Looking at other studies, Asghari et al. (2020), investigated drought resistance and biosynthesis stimulation of secondary metabolites caused by PGPR applied to mint plant limited water conditions. In the study, PGPR (*Azotobacter chroococcum, Azospirillum brasilense, Azotobacter chroococcum+ Azospirillum brasilense* and control) inoculation was determined as the first factor and three different (Field Capasity, 0.7 FC AND 0.4 FC) levels of irrigation regimen as the second factor. It was reported that chlorophyll content decreased significantly with drought stres, while antioxidant enzyme activity, MDA, abscisic acid and essential oil contents increased despite water limitation. Chemical fertilizers that are used unconsciously and for a long time cause serious damage to the plant and ecosystem (Kandpal, 2021). Considering the positive effects of organic and biofertilizers on the natural environment, the demand for these fertilizers has also increased (Alfarisy et al., 2021; Bhunia et al., 2021; Leno et al., 2021). It is known that plants inoculated with PGPR show tolerance to abiotic stres with morphological and bichemical modifications (Vurukonda et al., 2016). Santoro et al. (2015), reported that plants inoculated with PGPR strains increased shoot weight, number of branches and root dry weight compared to controls. The effects of plant growth promoting rhizobacteria on the anatomical, morphological and phytochemicals of mint (*Mentha piperita*) were investigated. As a result of the study, it was reported that plants inoculated with PGPR showed an increase in shoot and biomass, leaf area and stomatal density, and significant qualitative and quantitative changes in monoterpene content (Cappellari et al., 2015). Hassanpour et al. (2012), reported that growth and yield of *Mentha* plants were considerably reduced under water stress and that some PGPR applications under stress conditions could raise secondary metabolites and plant biomass.

2.2. Basil *(Ocimum basilicum* L.)

Among the *Ocimum* species belonging to the *Lamiaceae* family, the main species of economic importance is *Ocimum basilicum* L. Basil plant, which is originated from Iran, South Asia and especially India, grows naturally in Mediterranean climate and hot climates, and it is mostly cultivated in France, Italy and Spain (Akgül, 1993). *Ocimum basilicum* L. is known to have a wide morphological and chemical variation and being studied by dividing into subspecies and varieties. *Ocimum basilicum* L. and *Ocimum minimum* L. are cultivated for basil, which are not found naturally in the flora of Turkey. It is grown especially in Western and Southern Anatolian Regions. The economically used part of basil is usually the leaves. From its flowering, branches and leaves, 0.05-1% essential oil is obtained by steam distillation. The essential oil content and essential oil composition vary not only depending on the species, but also depending on the climate of the region where it grows, soil structure, harvest time, growth and development form of the plant, flowering and pigmentation. It is widely used as perfume, cosmetics, aromatherapy, traditional medicine and food flavoring due to its valuable essential oils and fragrances (Kulak, 2016).

Population growth and the increase in food demand is a global problem. Uncontrolled and excessive application of chemical fertilizers to increase agricultural productivity affects the World's water resources and causes environmental pollution. For the reason, it is necessary to switch to new applications that help to increase agricultural productivity. The use of plant growth promoting rhizobacteria (PGPR) is seen as promising in sustainable farming practices. PGPR are bacteria found in the rhizosphere that improve plant health by helping to increase plant growth (Goswami et al., 2016). Biofertilizers used in many plant groups have started to be used in medicinal and aromatic plants. In a study on basil, a medicinal and aromatic plant, the effects of PGPR on growth, essential oil and nutrient intake were examined. It was reported that the use of *Pseudomonas putida*, *Azotobacter chroococcum* and *Azosprillum lipoferum* bacteria increased fresh root weight, nitrogen content and essential oil yield (Ordookhani et al., 2021). Kırıcı et al. (2017), investigated the effect of bacterial isolates on yield and quality in basil and reported that the highest green herb yield, plant height and number of branches por plant were obtained from the treated with bacteria, while the lowest was obtained from the control group. In the another study, they reported that they achieved significant increases in plant height with the combined use of nitrogen fixing *Azotobacter a*nd *Azospirillum* bacteria in basil (Tahami et al., 2017). Khalediyan et al. (2020), investigated the effect of rhizobacteria and arbuscular mycorrhizae on growth, nutrient uptake and essential oil production of *Ocimum basilicum* and *Satureja hortensis* plants. In the study, they reported that the use of these fertilizers increased plant height, root duration, fresh and dry weights of shoots and their lenghts, shoot branching and inflorescence retention rate per plant.

Heidari and Golpayegani (2012), investigated the effect of PGPR on antioxidant and photosynthetic pigments in basil plants treated with three water levels (80%, 60% and 40% FC). At the end of the study, it was reported that PGPR applications increased antioxidant and photosynthetic pigments under water stress. Priority targets for mitigating the negative effects of drought stress include the implentation of easy and low-cost methods. The development of varieties that tolerate adverse environmental conditions is known as one of the most effective methods. However, the development of varieties with high tolerance to abiotic stress conditions such as drought is complex and time consuming. For this reason, the number of researches on the possibilities of using plant growth promoting bacteria (PGPR) to increase yield and to enable the plant to cope with stress conditions is increasing day by day (Marulanda et al., 2009). However, the main goal is to ensure sustainability in the cultivation of palnts by biological means without harming humans and the environment. Among the strategies used against water stress, the use of plant growth promoting bacteria has gained an important place thanks to its sustainable and versatile properties. Özmen (2023), investigated the effects of biofertilizer applications under water stress on the morphological, physiological and yield parameters, essential oil content and components and antioxidative enzyme activities of basil (*Ocimum basilicum* L.). In the study, it was reported that co-application of vermicompost with fixing nitrogen *Pseudoalteromonas tetraodonis* and fixing phosphorus *Brevibacillus choshinensis* bacteria was an effective and sustainable method against water in basil cultivation. Another study reported that PGPRs applied to the basil plant under salt stress, which is another stress factor, increased the number of branches (Agami et al., 2016).

2.3. Sage *(Salvia officinalis)*

Medicinal sage, a perennial herb from the *Lamiaceae* family, originates from the Mediterranean Region and European countries with coasts. *Salvia* species, which have been known by people since ancient times and used for their beneficial effects, are among the medicinal and aromatic plants that have an important place in the world. Although it does not have a natural distribution in our country, it is cultivated in various regions. Sage, which has many uses, especially an the pharmaceutical, food, cosmetics and perfumery sectors, has an important place among the medicinal and aromatic plants of Turkey (Öztürk et al., 2017). There are approximately 3 species belonging to the genus *Salvia* in the European contient, 70 species in Iran and 75 species within the former Sovet bordes (İpek and Gürbüz 2010). It is reported that there are 97 species of sage plants in Turkey, 51 of which are endemic (Güner et al., 2012). Medicinal sage (*Salvia officinalis* L.), apple sage (*Salvia pomifera* L.) musk sage (*Salvia sclarea* L.), Anatolian sage (*Salvia fruticosa* Mill.) Spanish sage (*Salvia lavandula efolia* Vahl.) are among the species with high commercial value (Angerhofer, 2000). One of the species, medicinal sage (*Salvia officinalis* L.), is not naturally distributed in Turkey (İpek and Gürbüz, 2010). However, this plant can be cultivated in our country (Karakuş et al., 2017).

Essential oils contain important bioactive substances (Katar et al., 2018). Medicinal sage leaves contain between 0.5-2.5% essential oil (Ceylan, 1996). Sage essential oils and exracts are used in the treatment of many diseases. Salvia species have various effects such as pain relief, facilitating digestion and preventing sweating (Topçu, 2006; Saydam, 2018). Studies have shown that sage essential oil can improve memory and be used in the treatment of Alzheimer’s disease (Perry et al., 2005). The word “salvia”, which gives sage its genus name, comes from the word “salvare”, which means to heal. Sage, which has varying amounts of essential oils, contains important essential oils such as α-*pinene, β-pinene*, *borneol*, *camphene*, *camphor*, *cineole* and *thujone* (Kutlular, 2007).

While the aim in herbal crops is to obtain high yields from unit area, it is to obtain higher quality products in medicinal and aromatic plants. In medicinal and aromatic plants, the active substance content is especially important. By determining suitable climatic conditions and cultivation techniques, suitable varieties with desired characteristics can be developed. Fertilization and maintenance processes are more important in such plants. In recent years, the tendency towards organic fertilizers has increased due to the fact that they are offered for consumption in various forms and their active ingredients are utilized (Özer, 2021). Biofertilizer applications, which are organic fertilizers as an alternative to intensively used chemical fertilizers, have also been used in sage. When the studies on the use of PGPRs in sage are examined, it is seen that positive results have been obtained. Underground microbial communities have a positive effect on the plant by reducing the effects of abiotic stress (Lau and Lennon, 2011). In a study investigated PGPR (*Bacillus megaterium*, *Enterobacter* sp., *Bacillus thuringiensis*, *Bacillus* sp.) applications on *Lavandula* dendata and *Salvia officinalis* plants under droght conditions, it was reported that bacteria improved the nutrition, physiology and metabolic activities of plants (Armada et al., 2014). Anbi et al. (2020), in their study on how PGPRs affect photosynthetic capacity and nutrient uptake in different *Salvia* species reported that bacteria alone and in combintion play an important role in plant growth and nutrient uptake. PGPRs have started to be used for many beneficial aspects such as increasing the quality and yield of by increasing the plant's uptake of mineral substances from the soil, protecting the plant from the damages of synthetic fertilizers, and increasing its resistance to adverse environmental conditions (Qiu et al., 2019; Khan et al., 2021). The effects of mineral, nano and bio nitrogen fertilizers on the nitrogen content of the plant and plant productivity were investigated in *Salvia officinalis* L. plant. In the study urea, nanourea and *Azotobacter chroococcum* applications were used as biofertilizers. With the application of increasing nanourea and urea fertilizer, the yield components of sage increased, and it was determined that the most effective results were the application where nanourea and biofertilizer were used together (Hegap et al., 2018).

4. Balm *(Melissa officinalis)*

A member of the *Lamiaceae* family, balm is a perennial plant and can grow up to about a meter tall. *Melissa officinalis* plant contains 0.06-0.3 % essential oil. The essential oil components it contains are linalool, nerol, citronellal, β-caryophyllene and β-caryophyllene oxidene (Mrlianova et al., 2001; Patora et al., 2003). Due to the essential oil components it contains, its taste and odor resemble lemon (Coşge, 2006). It is a medicinal and aromatic plant that can be used as antimicrobial, antioxidant and antiseptic due to its essential oil components. This plant, which can be used as fresh and dried leaves, is used as a sweetener, flavoring and spice in various foods and beverages. It is used in the treatment of nervousness and tension, depression and attention deficit, sleep disordes (Araujo et al., 2003).

Water scarcity is a serious threat to *Melissa officinalis* plant in arid and semi-arid regions which reduces global crop yield and essential oil production (Bruce et al., 2007; Moradkhani et al., 2010; Jamal Omidi et al., 2018). Plants develop some physiological and biochemical strategies in case of stress and produce some compounds to cope with stress (Yang et al., 2010). It is reported that the essential oil content and components of the plant are affected by factors such as drought stress and fertilizer use (Min et al., 2005; Amuamuha et al., 2012). *Melissa officinalis* is one of the most important medicinal and aromatic plants of the *Lamiaceae* family, which is affected by different irrigation regimes and bacterial inoculation (Gorgi et al., 2022). Positive results can be obtained in stress situations with PGPR applications. Kazeminasab et al. (2016), investigated the effect of vermicompost and PGPR on the physiological characteristiscs of *Melissa officinalis* L. under drought stress. As irrigation level, 100% field capasity and 60% field capasity were used. Vermicompst was used at 0, 0.5 and 1 t/ha and as biofertilizer, bacterial strains of *Pseudomonas fluorescent*, *Azotobacter chrococum*+*Azospirillum basilense*, *Azotobacte*r+*Azospirillum*+*Pseudomonas* were used. As a result of the study, it was observed that water stress increased the essential oil content and decreased the essential oil yield, total chlorophyll, relative water content and proline. It was reported that the relative water content and chlorophyll were positively affected in the application of biofertilizers. In a study conducted under greenhouse conditions, mycorrhiza and PGPR were applied to *Melissa officinalis* plant under drought stress. It was determined that the chemical composition of the secondary metabolites and the essential oil ratio in the leaves increased in the plant under drought stress with microbial applications. It was reported that PGPR+AMF inoculation provides increased yield in plants grown under limited water conditions (Gorgi et al., 2022). The use of growth promoting rhizosphere bacteri (PGPR) as biofertilizer in order to reduce the damage caused by environmtal stress is accepted as one of the new solutions in sustainable agriculture in arid and semi arid regions of the world (Nikbaht et al., 2020). Nikbaht et al. (2020), reported that bacterial applications inoculated into *Melissa officinalis* plant under limited water conditions reduced the negative effects caused by stress.

2.5. Rosemary *(Rosmarinus officinalis)*

Rosemary *(Rosmarinus officinalis* L.), known by different names such as Kuşdili, hasalbal and akpüren in Turkish, is an important medicinal and aromatic plant from the *Lamiaceae* family (Begum et al., 2013). Rosemary plant is a perennial plant with a height of 50-100 cm, shrub appearence, evergreen leaves in winter, pale blue flowers (Baydar, 2020). Rosemary plant can be shown as a growing area where the Mediterranean climate prevails. Rosemary has a wide range of uses in the world as an ornamental, aromatic and medicinal plant. Rosemary, which is usually collected from nature, has a large growing area in Turkey, France and Spain. It grows in the Western coastlineand Southern regions of Turkey (Kırıcı, 2015).

Rosemary essential oil is particularly valuable in perfumes, cosmetics and aromatherapy. Rosemary leaves contain between 0.3-0.9% essential oil under culture conditions and between 1.0-2.5% under natural conditions. The main components of the essential oil and exract from rosemary differ. When the studies on the antioxidant effective components, ratios and activities of rosemary are examined, it is noteworthy that the results differ according to the season, regions, the part of the plant used, the method of obtaining and the solvent used in the exraction. Apart from these, it is reported that genetics, water, light and vegetation period are also effective (Del Bano et al., 2003).

Soil quality largely depens on maintaining optimum amounts of soil organic matter and biological nutrient cycling (Ghosh et al., 2015). Soil bacteria are known to improve soil health and plant productivity by increasing nutrient availability in the rhizosphere (Lacava and Azevedo 2013). They reported that in soil inoculation with a mixture of bacteria, plants are fed more balanced and there is a main mechanism interaction betwen phosphate solubility and nitrogen uptake, nitrogen and phosphorus uptake improves root (Belimov et al., 1995; Sharma, 2002; Abo-Aly et al., 2006). Various studies have shown that commercially available biological fertilizers are effective in the growth of rosemary plant (Sadegh Kamaei et al., 2019; Hammam et al., 2021). In another study, it was reported that *Bacillus suptilis* and *Pseudomonas aeruginosa* bacterial strains were effective in the growth and development of *Rosmarinus officinalis* L. (Sharma et al., 2021). Leithy (2000), reported that *Azotobacter* biofertilization increased the amount of essential oil in rosemary (*Rosmarinus officinalis* L.). It was reported that the treatment of rosemary plant with microorganisms increased the growth characteristics and chemical content of the microorganisms compared to conventional (NPK) fertilization (Abdelaziz et al., 2007). Hammam et al. (2021), investigated the effect of biofertilization (algae, mycorrhiza and PGPR) on rosemary production under restricted water conditions. In the study, it was reported that PGPR applications both alone and in combination with other biofertilizers increased plant growth parameters at full irrigation. They reported an increase in plant oil content with PGPR inoculation in applications with limited irrigation. Denghani et al. (2019), who investigated the changes in essential oil in rosemary plants due to salinity stress and PGPR effects, reported that PGPR applications increased essential oil yield in plants even under saline conditions. Gachkar et al. (2007), reported that *Pseudomonas flourescence* bacterium has immune-boosting effects on the amount of essential oil composition by promoting antibacterial, antioxidant, antiviral stimulation in *Rosmarinus officinalis* L. plants.

3. CONCLUSION

The increase in the demand for food with the growing population in agricultural areas has led to the use of more chemical inputs. The use of rhizobacteria applications that promote plant growth is increasing day by day in terms of increasing crop yield quality and reducing the cost of synthetic fertilizers. Biofertilizers have shown positive results in reducing the cost of conventional fertilizers and the potential for environmental risk due to overdosing. Rhizobacteri have also been used in the cultivation of medicinal and aromatic plants and positive results have been obtained. Risk factors are minimized with PGPRs used even under stress conditions. The use of biofertilizers, which are produced, used and expected to be used more intensively in the future in medicinal and aromatic plants is stil very new in Turkey and studies are limited. PGPR should be used in medicinal and aromatic plants, taking into account the environmental effects as well as obtaining high yields from the unit area. Considering the advantages of biofertilizers from the soil-plant system, studies in this area should be intensified. Considering the number of studies, it is clearly seen that studies on the subject should be increased. This study has the potential to guide scientists who are interested in this field and want to work in this field, and to create a thought infrastructure.

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CHAPTER 7

TISSUE CULTURE OF ORNAMENTAL SUCCULENT PLANTS

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1. Introduction

Scientists and gardeners are interested in succulent plants due to their huge, juicy leaves and value to horticulture and commerce. Cacti are adapted to grow and produce under low water regimes and poor soils. The development of effective cactus propagation techniques over the past few decades has made species considerably more widely available. Cacti have seen a remarkable rise in cultivation in recent years. Cacti, like many other decorative plants developed for commerce, can be multiplied sexually and vegetatively. The process of growing cacti from seeds takes time and requires particular lighting and temperature conditions. In some cases, seed germination percentage is low due to the physical dormancy imposed by the hard coat.

The accumulation of a sizable volume of releasable water in living cells is referred to as succulence. It is dependent on changes in quantitative factors such tissue thickness, cell volume, and cell packing. Three to five percent of all flowering plants are often classified as succulents (Griffiths & Males, 2017). Estimates of the succulence's taxonomic range include up to 83 plant families and 12,500 species (Nyffeler & Eggli, 2010).

Succulents have always been seen as distinct despite their odd morphologies, which have been variably described as grotesque monstrosities and exotic curiosities. One of the most notable instances of convergent evolution in the plant kingdom is thought to be the succulent syndrome. Large cells used to store water are present in all succulents. The level of cellular succulence in roots, stems, and leaves is influenced to some extent by evolutionary coordination, but cellular succulence can occur in every vegetative plant organ. Succulence occurs in plants that have evolved in a variety of habitats and is linked to a wide range of ecophysiological strategies (Griffiths & Males, 2017).

Biologists have long been fascinated by succulent leaves. The definition of succulence, the evolutionary history, the effects of leaf succulence, and the ecological significance of water storage for plants living in arid environments have all been extensively studied. Despite improvements in our knowledge of the molecular foundations of leaf architecture in model systems, little is known about the molecular regulation of leaf succulence. Leaf succulence is also a drought resistance feature that hasn't been extensively utilized for crop improvement (Heyduk, 2021).

The primary source of water storage is in simple parenchyma cells, which have a vacuole that may occupy more than 95% of the cell volume (Antony et al., 2008). While the hydrenchyma in certain succulents is non-photosynthetic and distinct from the outer mesophyll layer of the succulent sections, in all-cell succulents (such as Crassula), these cells contain chlorophyll and contribute to the photosynthetic burden of the succulent organ (e.g. leaves of Aloe). The vacuole serves as a storage space for metabolites like sugars and is important for maintaining pressure inside the cell and the general stiffness of the tissue (Grace, 2019).

2. Ornamental Succulent Plants

Succulents are becoming more attractive plants. This is mainly due to their distinct geometrical shapes, which form a rosette, as well as their capacity to hold high amount of moisture. Because of these qualities, attractive succulents are appropriate as both potted plants for indoor settings and landscape plants because they can tolerate harsh circumstances (Cabahug et al., 2018).

The *Mammillaria* (93 species), *Opuntia* (47 species), *Coryphanta* (37 species), and *Echinocereus* (35 species) genera account for 2/3 of commerce in the world market for cactus species. Also *Hamatocactus crassihamatus*, *Geohintonia mexicana*, *Isolatocereus dumortier*, *Neolloydia conoidea*, *Leuchtenbergia principis*, *Obregonia denegrii* and *Strombocactus disciformis* species involved in the international market. According to Barcenas-Luna (2003), *Aztekium* and *Lophophora* each have two commercial species (*Astekium ritteri* and *A. hintonii*; *Lophophora williamsii* and *L. diffusa*).

Fig. 1. Sedum 'Sun Red' succulents (A: 4,000 lux and 3 hours, B: 8,000 lux and 3 hours lightning duration and intensity) (Nam et al., 2016).

The most often seen ornamentals in the USA are from the genera *Mammillaria* and *Opuntia* (*O. phaecantha*, *O. engelmannii*, *O. violacea*, *O. basilaris*, and *O. ficus-indica*). The candelabra cactus (*Cereus peruvianus*), also known as Christmas or Thanksgiving cactus, and *Schlumbergera truncata* are two other common species (Irish, 2001). In northern Europe, the genera *Schlumbergera* and *Hatiora* are important glasshouse crops that are commonly grown as flowering potted plants. *Hatiora gaertneri*, *H. rosea* and their interspecific hybrid are commonly known as Easter or holiday cacti (Pérez-Molphe-Balch et al., 2015).

Fig. 2. Flowers of Cacti and Succulents (Roy & Khuraijam, 2015)

3. Propagation of Ornamental Succulent Plants

Historically, members of the *Cactaceae* family were primarily multiplied vegetatively through cuttings and grafting or by seeds (Lema-Rumiska & Kulus, 2014). Succulent ornamental plants can be multiplied in a variety of ways, including sexually through seeds and vegetatively by stem cuttings, leaf cuttings, and micropropagation. Typically, there are two primary groups of succulent propagation techniques: sexual propagation and asexual propagation. It is uncommon to reproduce succulents from seeds. In asexual or vegetative propagation, stem and leaf cuttings are used, along with methods for separating suckers and rhizomes. In vitro culture or micropropagation is a quicker technique that is nevertheless regarded as vegetative propagation (Cabahug et al., 2018).

The most popular cactus micropropagation techniques are axillary bud activation, adventitious shoots, and somatic embryogenesis regeneration. According to Lema-Rumiska and Kulus (2014), these methods have been used with a variety of cactus taxa, including *Astrophytum*, *Cephalocereus*, *Aztekium*, *Cereus*, *Coryphantha*, *Copiapoa*, *Echinocactus*, *Ferocactus*, *Echinocereus*, *Gymnocalycium*, *Mammillaria*, *Leuchtenbergia*, *Nyctocereus*, *Rhipsalidopsis*, *Opuntia*, *Schlumbergera* and *Stenocactus*.

4. Direct Organogenesis (Activation of Axillary Buds)

In vitro organogenesis is the method of producing organs from beginning using cells, tissues, or organs that have been cultivated under sterile, controlled circumstances. Organogenesis can take place directly from cells, tissues, or organs in culture or it can occur in a more indirect way after the intermediary steps of callus formation (de-differentiation), which leads to the development of new organs. Shoot development and root induction are typically the two phases involved in in vitro plant regeneration via organogenesis. Direct organogenesis is the technique of producing new buds directly from tissue without the need for a callus stage in between. Direct organogenesis micropropagation has been routinely used over the past few decades to secure the micropropagation of numerous endangered species, including *Cactaceae* family species (Bouzroud et al., 2022).

The growth of plants from lateral meristems and proliferation are required for axillary bud activation. After that, the shoots can be separated and planted in a rooting media. Cacti have latent buds in their areoles that could become active in the right circumstances (such the presence of specific PGRs). Numerous axillary shoots are produced by activated areoles (Lema-Rumiska & Kulus, 2014). By activating axillary buds, many cacti have been multiplied. *Mammillaria elongata*'s typical shape was reproduced in culture by growing callus from tubercle explants that were removed from the branch's uppermost portion and grown on MS medium containing NAA and BA (Papafotiou et al., 2001).

For *Pelecyphora aselliformis* and *P. strobiliformis*, Pérez-Molphe-Balch and Dávila-Figueroa (2002) established an effective in vitro propagation technique. The average rooting efficiency for *P. aselliformis* and *P. strobiliformis* was 89% and 87%, respectively. Once planted in soil, the plants typically survived 88% of the cases.

In vitro propagation systems by means of areole activation were developed by Dávila-Figueroa et al., (2005) for *Turbinicarpus laui, T. pseudopectinatus,* T. *lophophoroides*, *T. schmiedickeanus* subsp. *flaviflorus*, *T. schmiedickeanus* subsp. *schmiedickeanus*, *T. schmiedickeanus* subsp. *klinkerianus*, *T. subterraneus*, and *T. valdezianus*. Explants were primarily obtained from seedlings that were in vitro-germinated. Three explant types (apical, lateral, and transverse) cultivated on MS basal medium supplemented with sucrose, agar, and numerous treatments with cytokinins. Multiple shoot development from areoles was accomplished. The greatest results in terms of explant type were achieved with transverse incisions for five species, apical explants for one species, and the two remaining species exhibited no significant differences among the explants evaluated. On half- or full-strength MS basal media, rooting of the in vitro-generated shoots was most successful.

A methodology for the in vitro culture and plant regeneration of *Notocactus magnificus*, the blue cactus, a highly decorative species native to Brazil, was developed by de Medeiros et al., (2006). The MS medium that was treated with benzylaminopurine, sucrose, and agar produced the most shoots. After eight months of cultivation on MS media, in vitro spontaneous rooting of shoots was seen. Only in vitro rooted shoots maintained normal plant development in glasshouse culture.

Shoots by direct and indirect organogenesis and somatic embryos were induced from tubercles excised from *Ariocarpus kotschoubeyanus* seeds germinated in vitro by Moebius-Goldammer et al., (2003). The highest number of shoots (6.3 per explant) formed when explants were grown on MS medium with BA and NAA supplements. Individualized shoots were rooted in half-strength MS; the rooting of shoots was enhanced by the inclusion of activated charcoal and the application of sun cap closures to seal containers. On media containing mixtures of BA and NAA, about 20% of the explants generated somatic embryos.

Ramirez-Malagon et al., (2007) conducted in vitro propagation for ten *Mammillaria* species and tested 20 combinations of IAA and kinetin. Best results on shoot formation were obtained using kinetin at two levels. All IAA levels tested were able to induce de novo shoot formation in *M. bocasana, M. hahniana, M. densispina, M. hutchisoniana, M. pectinifera, M. orcutii, M. perbella, M. rhodantha, M. picta*, and *M. zephyranthoides*. Four responding groups were examined for their highest shoot-formation number, according to the IAA level tested. With 5.7:46.5 or 11.4:46.5 M IAA/kinetin, giving 4.8 and 4.7 shoots per explant, respectively, in 60 days, was the ratio that produced the highest average number of shoots for all species. The explants were either left in their shoot-induction medium or moved to half-strength MS medium to allow the regenerated shoots to take root.

Rubluo et al., (2002) conducted observations on the influence of auxins as the sole exogenous growth regulator on the morphogenesis of long-term in vitro subcultured plantlets of the severely endangered cacti *Mammillaria san-angelensis*. Auxin concentration was found to have a direct impact on plant regeneration after sections of long-term subcultured shoots were exposed to various auxins at varied concentrations. It was discovered that morphogenetic potentiality was preserved in long-term subcultures and that IAA (34.25 M) promoted the best regeneration.

In a work by Retes-Pruneda et al., (2007) areole activation was used to develop in vitro growth systems for *Echinocereus knippelianus*, *E. schmollii*, *Mammillaria carmenae*, *M. herrerae*, "M. *carmenae* fo. *rubrisprina*", *M. theresae*, *Escontria chiotilla*, and *Melocactus curvispin*. Seedlings which has germinated in vitro were used as explant sources. On MS basal medium with 3% sucrose, agar, and BA or 2iP added as supplements, multiple shoot development from areoles was accomplished. *M. carmenae* fo. *rubrisprina* had an efficiency of 6.0 shoots per explant, while *Echinocereus schmollii* had a productivity of 13.5. In MS basal medium or MS basal medium enriched with indoleacetic acid, indolebutyric acid, or activated charcoal, rooting of the in vitro produced shoots was accomplished.

Quiala et al. (2009) established areole activation-based in vitro propagation systems for *Pilosocereus robinii*. Seeds from mature fruits were collected under field conditions and disinfected. The use of half MS basal medium resulted in the highest germination rate (92.8%). On average, rooting efficiency was 100% in MS basal medium free of growth regulators.

For the prickly pear cactus (*Opuntia ficus-indica*), El Finti et al. (2012) attempted to develop a mass and quick micropropagation method. On MS medium containing phytagel, BA, sucrose, monosodium phosphate, and adenine sulfate, young cladode explants with one areole were grown. The best rates of multiplication were found with BA as opposed to kinetin. The multiplied branches were moved to a medium containing half MS, sucrose, phytagel, and several auxins to encourage roots. In these cultivation circumstances, IBA and IAA produced the largest number of roots (21.05 and 16.2) and 100% of plantlets that rooted.

5. Somatic embryogenesis

Regeneration of the entire plant from the plant cell during embryo formation was found to be an alternative to root and shoot regeneration from the callus. Somatic embryogenesis is the process through which a vegetative or somatic cell develops into an embryo or a plant. A method of plant regeneration known as somatic embryogenesis involves the development of embryos from somatic cells. Similar to zygotic embryos, these embryos can germinate and grow into a whole plant. Regenerated plants, however, still have the genetic makeup of the original plant because they come from somatic cells. Either directly on the explant or indirectly via calli, somatic embryos can be formed. This is one of the most efficient ways for in vitro mass propagation of plants. However, many species are recalcitrant and very difficult or impossible to regenerate in this way (Pérez-Molphe-Balch et al., 2015).

From phylloclade explants of "*Schlumbergera truncata* cv. Russian Dancer," somatic embryogenesis was produced. In comparison to callus grown for a shorter length of time in the establishing medium, callus developed on phylloclade explants and subcultured over a period of 16 months on MS medium containing mostly cytokinins was more effective for the induction of somatic embryos. When utilized as the first medium to induce somatic embryogenesis, SH-based medium outperformed MS-based media. However, when 2,4-D was added to the MS-based medium used for final transfer compared to the medium without growth regulators, somatic embryogenesis, as opposed to adventitious shoot production, was reduced, indicating that a crucial hormonal balance had been established. When cultured on G medium, somatic embryos produced root and shoot poles. On this medium approximately 70% germination was recorded in the embryos that were differentiated earlier from the callus that was grown for a longer time in the establishment medium (Al-Ramamneh et al., 2006).

An in vitro protocol has been developed for callus induction, somatic embryogenesis, and plant regeneration in the Barbary fig (*Opuntia ficus-indica*) cultivars ‘Gialla’ and ‘Moore’. Primary nodular and greenish calli were formed by immature anthers grown in the dark on induction medium made up of Chée and Pool media containing 2,4-D and thidiazuron during 6 to 8 weeks. After 4 to 5 months of cultivation, periodic transfer of nodular calli onto induction media in the dark resulted in the creation of compact embryogenic masses. On induction medium, somatic embryos went through a number of diverse stages of development, including secondary somatic embryos and pro-embryogenic, globular, heart-, torpedo-, and cotyledonary-shaped somatic embryos. Further development of somatic embryos was accomplished using half-strength

Murashige and Skoog medium containing activated charcoal. Regenerated plantlets with well-developed cladodes and roots (Bouamama et al., 2011).

In vitro segmentation of shoots was used to generate *Schlumbergera truncata* plants, which were then duplicated and rooted. On MS nutrient medium supplemented with BAP and NAA under the conditions of placement on the nutrient medium and performing a significant number of cuts on the explants, ideal conditions for the induction of callus formation in stem node segments of *S. truncata* plants (rate more than 90% and significant growth) were produced. Statistics show a statistically significant relationship between the frequency of callus formation and the light regime and nutrient medium composition. On the MS medium, adding BA resulted in the greatest number of shoots. At the same times, shoot proliferation and root induction in such numbers were observed on MS culture medium with the addition of BA and kinetin (Chornobrov & Bilous, 2021).

In cactus pear, embroyogenic process was induced from generative explants consisting in ovule, extracted from flowers, 10 days after anthesis by Jedidi et al., (2013).

6. In vitro Grafting

The process of grafting a micro-scion onto the stems of young rootstock plants produced through in vitro culture is known as in vitro grafting. For cactus, this propagation method has been used as an alternative to grafting since it has less problems with poor success, contamination concerns, and dehydration stress of the tissues around the graft union (Estrada-Luna et al., 2002). One of the limits of micropropagation is the species-specific requirement of plant regulators for each taxon and sometimes even for different genotypes. The necessary hormonal requirements for the scion are directly provided by the rootstock during the micrografting procedure (Badalamenti et al., 2016).

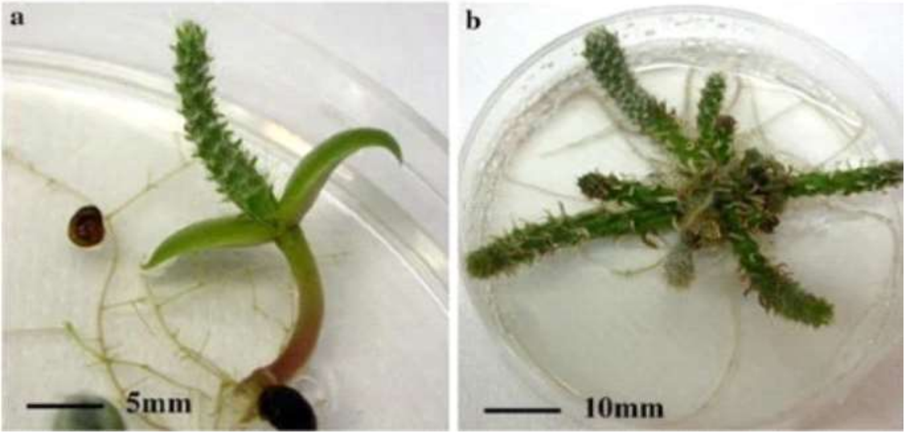

Fig. 3. Different stages of micrografting procedures: a) Seedling of Opuntia ficus-indica 30 days after sowing; b) Opuntia ficus-indica subcultured and maintained as rootstock source indefinitely (Badalamenti et al., 2016)

Cacti species are frequently reproduced by the use of grafting. *Gymnocalycium mihanovichi*i is an ornamental plant and should be grafted to root stock containing chlorophyll. Exogenous auxin treatments were used in this study to enhance grafting. *Trichocereus spachianus* and *G. mihanovichii* were employed as the root stock and scion, respectively. As an auxin, IBA was used. Plants were exposed to four distinct IBA concentrations (0, 50, 100, and 150 ppm), which were repeated three times (3, 9 and 15 days after micrografting). IBA's ideal concentration was 100 ppm, especially after three repetitions. According to the study's findings, IBA is a successful treatment that can enhance the number of successful grafts when used at the right concentration (Moghadam et al., 2012).

To find the most effective technique for in vitro micrografts, micropropagated prickly pear cactus (*Opuntia* spp.) was used with horizontal and wedge grafts (Estrada-Luna et al., 2002). The horizontal graft was shown to be the simplest and most effective technique for micrografting prickly pear cactus, according to the results. In all genetic combinations, vascular linkages between the rootstocks and scions formed within the callus bridge within 28 days. The stock-scion combinations of the *Opuntia* species were hence compatible. After 90 days of ex vitro transfer, homografts (grafts between members of the same species) expanded noticeably more quickly than heterografts (grafts between members of different species). The development of a necrotic layer, the proliferation of a callus bridge at the graft interface, the differentiation of new vascular cambium, the restoration of the new vascular tissue, and the restoration of the continuity of the external epidermal tissue at the union zone were all seen histologically as the five developmental stages of graft union formation.

7. Indirect Organogenesis (Callus Culture)

Indirect organogenesis is the process by which plant organs develop from a calli mass (tissue build up on a plant wound or at the site of a cut) in the explant. A callus is an undifferentiated tissue that forms as a result of tissue damage and/or uncontrolled cell growth. This tissue has been employed as a stepping stone toward indirect plant regeneration via organogenesis or somatic embryogenesis since it is simple to acquire and sustain in vitro. The callus develops from the in vitro proliferating cells of plant tissue in response to biotic and abiotic stimuli. It is a mass of loosely packed parenchymatous cells with varying degrees of differentiation. Indirect organogenesis has been successfully implemented for several cacti species such as *Ariocarpus kotschoubenyanus*, *Astrophytum asterias*, *Blossfeldia liliputiana*, *Coryphantha elephantidens*, *Strombocactus disciformis*, *Mammillaria elongata*, *Mammillaria mathildae*, *Mammillaria pectinifera*, *Mammillaria gracilis*, *Escobaria minima*, and *Pelecyphora aselliformis* (Bouzroud et al., 2022).

Escobaria minima, *Mammillaria pectinifera*, and *Pelecyphora aselliformis* are three species of cactus that are in danger of extinction. Giusti et al., (2002) examined the viability of in vitro culture techniques for their proliferation. Despite the profuse callus development and hyperhydricity of axillary shoots, TDZ resulted with a good proliferation rate. On media containing BA for "*E. minima*" and "*M. pectinifera*" and on a medium containing kinetin for "*P. aselliformis*," a high multiplication rate along with acceptable quality proliferating branches and minimal to no callus induction were seen.

Using coconut water and complete darkness, del Socorro Santos-Daz et al., (2006) proposed a technique for micropropagating *Ariocarpus kotschoubeyanus*. On Murashige and Skoog medium (MS) with 2 mg/l zeatin, the longitudinal explants from seedlings that had germination in vitro were grown. The resulting calli were then transferred to a medium containing 5 ml, 10 ml, or 15 ml of coconut water per litre. After 84 days, each callus produced five green shoots during photoperiod but 14 well-defined shoots under darkness.

García-Rubio & Malda-Barrera, (2010) established a rapid shoot multiplication protocol for the endangered cactus *Mammillaria mathildae*. Explants were derived from seedlings that had been in vitro-germinated. Apical, lateral, and basal explants taken from in vitro-germinated seedlings were investigated as three explant sources. By adding various BA/IAA combinations to MS media, shoot multiplication was induced. In the presence of any BA/IAA concentration, explants produced an abundance of callus. Both apical and lateral explants generated new shoots without callus formation.

Results of the production of sterile cultures and micropropagation of *Astrophytum asterias, Blossfeldia liliputiana*, and *Strombocactus disciformis* were revealed by Ivannikov et al., (2022). It was established that using stems from young, in vitro-grown plants was an efficient way to propagate these cacti species. The ability to obtain the required amount of plant material by indirect organogenesis is demonstrated. For all stages of the micropropagation, the appropriate nutrient medium composition and favorable growing conditions were authorized.

Shoot cultures of many species of cacti have been obtained using either seeds or shoot fragments as starting materials (Malda et al., 1999). According to Vyskot and Ja'ra (1984), cacti have a special kind of latent buds called areoles that are composed of groups of spines or other appendages that can be activated in vitro to produce shoots. Alternatively, explant-derived callus may be used to indirectly produce shoots (Pelah et al., 2002), but this method is less preferred because it is more likely to cause genetic instability (Wyka et al., 2006).

The organogenetic potential of *Mammillaria albicoma* flower buds was examined by Wyka et al., (2006). Buds were incubated on solid MS medium supplemented with NAA and BA. Both inside the perianth and at the cut explant base, callus development was seen. These calli during subsequent subcultures to the same medium gave rise to adventitious shoots. Additionally, shoots developed right from the perianth. On transfer to a fresh medium, the shoots produced proliferating cultures.

The successful use of flowers and floral components as explants to begin tissue culture is rare. In the investigation by Wyka et al., (2009) flower buds of *Mammillaria albicoma, Mammillaria carmenae*, and *Mammillaria schiedeana* (*Cactaceae*) were cultivated on solid MS media containing NAA and 6-benzylaminopurine. All three species showed signs of developing shoots as well as vegetative buds (areoles). They found evidence for both direct and indirect mechanisms of shoot morphogenesis. Microscopic investigation of *M. albicoma* and *M. carmenae* demonstrated direct shoot morphogenesis from the perianth, particularly from the axils of the perianth segments. Explants that developed shoots were taken out and used to produce proliferating cultures. Proliferated shoots were then planted in vitro on MS media that contained 0.01 mg l-1 NAA and adapted to ex vitro conditions. Shoots from *M. carmenae* were also non-aseptically planted in horticultural substrate. Harvesting flowers from this group of succulents with leafless stems may be a practical approach to micropropagate desirable genotypes without harming stock specimens.

8. Conclusions

2/3 of the trade in cactus species on the global market is accounted for by the genera *Mammillaria*, *Opuntia, Coryphanta*, and *Echinocereus*. Only a few cactus species have been micropropagated for profit, and in some other instances, the main goal has been the proliferation of threatened or endangered species to rebuild their wild populations. Thus, the development of micropropagation methods for a large number of cactus species that must be multiplied for commercial purposes should be the main emphasis of future studies.

Vegetative propagation is impacted by cutting type and source, cicatrization time, and stem-segment juvenility. *Opuntias* (prickly pears, *Cylindropuntia*), columnar cacti (*Cereus* spp., *Trichocereus* spp., *Myrtillocactus, Hylocereus*, and *Lophocereus*), and a number of globular cacti are among the genera whose members can be grown through stem cuttings (*Echinopsis* spp., *Mammillaria* spp.). Genetic engineering and the production of haploid and double-haploid plants don't seem to have received much attention.

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CHAPTER 8

THE RISE OF WINTER CROP DRY PEA (*PISUM SATIVUM*) IN THE AGE OF GLOBAL CHANGE

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1. Introduction

Cool-season legume crop dry pea is known until Neolithic times and consumed by human and used for animal feeding after domestication approximately 9.000 years ago. In 2021, total 95 countries produced 14,2 million tonnes of dry peas on 6402 thousand ha land. Climate change and abiotic and biotic stress factors are increasingly threatining dry pea globally but until now the crop is defending itself good as observed reflections in yields and acreage increases of countries probably by the help of continuous worldwide genetic improvement activities.

Here in this review, a brief look to dy pea procution in the last decade was the subject with production statistics, some aspects of breeding activities and cultivation of dry peas.

Dry pea (*Pisum sativum* L.) is an important food since Neolithic times which was domesticated approximately 9.000 years ago and used for human consumption and as animal feed. Cool-season legume crop dry pea is produced worldwide in cool temperate climatic conditions (McPhee, 2003).

Total 95 countries were producing dry peas in 2021 in the world. Total world dry pea production amount has increased to 14,2 million tonnes in 2021 from value of 11,7 million tonnes in 2014. Highest grain yield values among top 20 producer countries were at UK (31992 hg/ha), Germany (30614 hg/ha) and İtaly (28588 hg/ha) in 2021 year. World total acreage for dry peas has increased to 6086 thousand ha in 2021 and from value of 6402 thousand ha in 2014 (Table 1) (FAOSTAT, 2023).

Table 1. Top 20 Country for Dry Pea Production Amounts, Yields and Acreages in 2021 and 2014 (FAOSTAT, 2023)

Rank	**Country**	**Grain Production (1.000 tons)**		**Grain Yield (hg/ha)**		**Acreages (1000 ha)**	
		2021	**2014**	**2021**	**2014**	**2021**	**2014**
1	Canada	2258	3810	15141	23726	1491	1605
2	Russia	3167	1503	22463	16756	1410	897
3	China	1467	1350	15693	14211	935	950
4	USA	388	778	11489	21376	337	364
5	India	876	925	14446	9605	606	963
6	France	552	539	28414	37101	194	145
7	Ukraine	566	359	23575	23390	240	154
8	Ethiopia	384	351	17334	14467	221	243
9	Germany	299	155	30614	37242	98	42
10	Romania	159	51	21225	18811	75	27
11	Australia	399	342	15863	13992	252	244
12	Spain	177	142	15416	10177	115	140
13	UK	195	128	31992	40000	61	32
14	Lithuania	121	101	19672	24719	62	41
15	Colombia	39	52	14369	17491	27	29
16	Kazakhstan	93	35	9525	7996	97	44
17	Argentina	194	145	22376	17059	87	85
18	Myanmar	33	49	7763	9100	43	54
19	Estonia	59	34	17873	20692	33	17
20	Italy	51	23	28588	23113	18	10
	World Total	**14,2**	**11,7**			**6402**	**6086**

Most of the countries among top 20 producers in the world has increased dry pea production amount in 2021 compared to 2014 but Canada, Colombia, Myanmar and USA were reduced.

Turkey's dry pea production was 2.200 tonnes in 2021 and 3.000 tonnes in 2014. Country wide yield was 26599 hg/ha in 2021 and 25997 hg/ha in 2014. Acreage of Turkey in 2021 was 602 ha and 1.150 ha in 2014. Acreage and production was lower but yield was higher in 2021 compared to 2014 in Turkey (FAOSTAT, 2023).

2. Genetics

Pea was model crop for genetic studies of Gregor Mendel. Pea is in *Leguminosae* family and consists of two species (*Pisum fulvum* and *Pisum sativum)*. The genetic diversity centre of pea is Fertile Crescent with probably Ethiopia as a secondary diversity centre (Warkentin et al., 2015).

Climate change shifts abiotic (high temperature, cold, frost, wind, drought) and biotic (pathogens, pests) stress factors and threats agricultural productivity around the world (Shahzad et al., 2015). As biotic stresses, fungal diseases of rust, powdery mildew, root rots, common root rot, wilt and ascochyta blight are the widespread and severe for pea crops at different growth stages. Among abiotic stresses, heat, drought and frost are frequent which reduce quantity and quality of dry pea grains. Genetic improvements for these traits are important and needed. Conventional and molecular breeding approaches may accelerate breeding programmes for improvements (Parihar et al., 2020).

Most of the breeding activities of peas are still conducted in public organisations in Canada, Australia, US, Europe, India and China. Smaller breeding programmes exist in Africa and South America. Private pea breeding companies are in Europe, US and New Zealand. Through breeding, progress in improving lodging resistance, disease resistance, visual seed quality and abiotic stress resistance (heat, salinity and frost) are targets. Pea breeding methods are pedigree, single-seed descent and F2-derived family methods. A pea genome sequencing study was just started by an international research consortium (Warkentin et al., 2015). Genetic resistance to overcome biotic factors (pathogens and insects) have been primarily introduced by biparental mating or backcrossing. Modern DNA technology was provided breeders new tools to study inheritance and improve breeding efficiency. To generate variation not present in germplasm, studies focus on mutagenesis and biotechnology. Biotechnology allow transfer of distant genes from unrelated germplasm into pea which are often supply resistance to diseases and harmful insects (McPhee, 2003). Although fungal and viral pathogens reduce seed yield and quality, resistance is available or incorporatable for many dry pea cultivars. Introduction of novel traits from distant landraces for resistance improvement can be achieved by backcrossing or biparental mating as well as cyclical hybridization. Mutagenesis can be used for genetic variation (McPhee, 2003).

Dry pea seeds are classified based on different morphological traits: Color (cream-yellow, yellow-green, light-green, green, army-green, dark-green, brown, orange-brown), shape (cylindrical, elliptical, rhomboid, irregular), surface (smooth or rough) (Fig. 1).

A study was conducted to see the relationship between yellow pigments of dry pea seeds and climatic conditions. Different colored three dry pea cultivars were grown under field conditions. Drought during seed development stage resulted with decrease in the xanthophyll content of seeds, significantly. A "yellow index" was defined as carotene/xanthophylls ratio, expressed yellow colour intensity of the dry pea seeds (Nemeskeri, 2006).

Fig. 1. Pea seed categories defined as colour, surface and shape (Santos et al., 2019).

3. Agronomy

Pea is grown in small farms as a pulse in South Europe where traditional old variety usage is common. In a study of Santalla et al., (2001), total 124 unimproved pea varieties and 10 elite cultivars were evaluated for dry pea agronomic traits. Significant G x E (genotype by environment) interactions were determined for protein ratio, fresh seed weight and size, pod weight and length, days to flowering, days to fresh seed and days to pod maturity (Santalla et al., 2001).

Dry pea seed dry weight and protein content are influenced from genetic and environmental factors. Seed development of dry peas at different main stem nodes within-plant were determined for six genotypes during field trial by Atta et al., (2004). Protein content were significantly increased from top to the bottom of the plants of three genotypes.

Dry pea requires early and effective management of weeds for higher yields. A single herbicide application is not able to control all weeds (Harker et al., 2007). Parasitic weed *Orobanche crenata* effect cultivation of dry pea strongly in Middle East and Mediterranean regions. Resistant cultivars does not exist but incomplete resistance was identified in wild relatives of dry pea (Fondevilla et al., 2017). *Didymella pisi* is the causal pathogen of ascochyta blight of dry pea causing yield losses in Montana, where 150.000 ha dry pea were planted in 2018 (Owati et al., 2019).

The Pacific Northwest is an important region for dry pea production in US (Zhang et al., 2021). Rolling soil following planting is a standard application for dry pea production in the Pacific Northwest (Porter & Coffman, 2007). Dry pea is in rotation with cereals in Pacific Northwest due to higher wheat yields after spring pea production (Clement, 2003).

Dry pea has potential to replace maize as an ethanol plant. Dry pea production costs are lower than maize. A study was conducted by Goel (2007), to evaluate replacement of dry pea with maize as ethanol source. Researcher determined that a 20% prices increase is needed for corn for dry pea to become competitive.

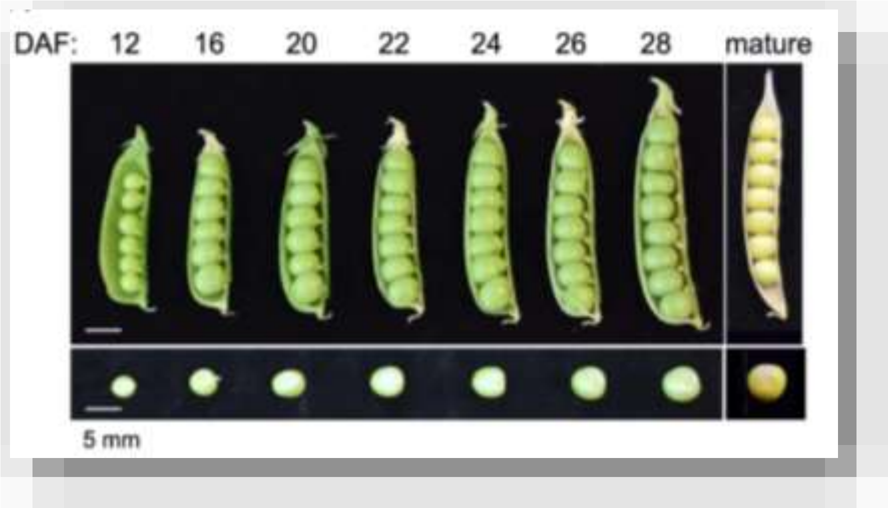

Fig. 2. Opened pods from 12 days after flowering (DAF) to maturity (Moore et al., 2018).

Fertilization requirement of dry pea is higher than forage pea (Karagic et al., 2007). To promote dry pea production in cereal based cropping systems, high yielding and well adapted genotypes are required for wide ranged environments (Mohammed et al., 2016). Grain yield stability of nine dry pea genotypes were tested in Croatia in two locations for three years by Cupic et al., (2008). Genotype (G), location (L), year (Y) and their GxL, GxY, LxY interactions were significant for grain yield of dry pea.

Nayak et al., (2011) investigated the phytochemical profiles and antioxidant activity of dry pea flour. Extracts in the free fraction contributed 87%, 86% and 64% to total phenolics, total antioxidant activity and total flavonoids, respectively.

Cooking time is an important criteria of dry pea food quality. Cooking time showed positive significant correlations with seed weight and cotyledon percentage and negative significant correlations with ash in a study of Ahmed (2006).

Pea resistant starch has received attention from food industries in recent years. In a study of Tao et al., (2017), grain yield varied between 980-5900 kg/ha, resistant starch content ranged between 5-55 g/kg and protein ranged between 160-250 g/kg. Analysis revealed that environment was the most important factor for grain yield, protein, and total starch whereas resistant starch content was mainly determined by cultivar.

In Serbia, Antanasovic et al., (2011) assessed performance of intercrops of normal-leafed ('Dukat') and semi-leafless ('Partner') dry pea cultivars at different plant densities (80, 100 and 120 plants/m2) and mixture ratios (semi-leafless:normal-leafed cultivars at ratios of 100%:0%, 75%:25%, 50%:50%, 25%:75% and 0%:100%). According to two years average, yield of semi-leafless cultivar was significantly higher than the normal-leafed cultivar. Highest land equivalent ratio value was obtained from intercrops of 75%:25% “semi-leafless”:”normal-leafed” cultivars.

4. Conclusions

Total world dry pea production amount has increased to 14,2 million tonnes in 2021 from value of 11,7 million tonnes in 2014. While production and cultivation area decreased in India, yield increased in 2021 compared to 2014. India was probably shifted to other profitable crops in this period when these data evaluated.

In Australia, acreages, yield and production increased compare to 2014. Similarly, in Argentina acreages, yield and production increased in 2021 compared to 2014. Germany experienced yield reductions due to 100% acreage increases.

As a conclusion, it can be said that world dry pea production is increasing instead of global warming probably due to the benefit of cultivation under winter conditions. It is logic for farmers to cover fields with crops in winter periods in next decades.

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CHAPTER 9

AUTOMATED HIGH-THROUGHPUT PLANT PHENOMICS FOR THE ANALYSIS OF GENOTYPE X PHENOTYPE X MANAGEMENT INTERACTIONS

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Introduction

The complex interaction of genetic information (G) with the multifactorial, abiotic, and biotic environment (E), and agricultural management regime (M) determines the plant phenotype. Linking phenotypes with genotypes to identify genetic architectures that regulate important traits is essential for plant breeding and the development of plant genomics. Breeders often largely miss crop processes, while crop physiologists frequently fail to understand the importance of varieties and cultivars on crop growth and development. to integrate the different levels of regulation involved in the expression of post-transcriptional, post-translational, and epigenetic mechanisms, the plant physiology serves as an essential link between the genome and the phenotype. One of the most recent innovations to enable non-destructive screening of plants over certain time intervals using image capturing techniques is an automated greenhouse system for high-throughput plant phenotyping.

Significant advancements in molecular and genetic plant breeding have been accomplished in recent years. The ability to profile the crop phenome, or the structure and function of plants in relation to allelic variants and environments, still remains a substantial technological challenge despite advances in genomics have greatly assisted plant breeding efforts (Tardieu et al., 2017). Linking phenotypes and genotypes to identify genetic architectures that regulate important traits is essential for plant breeding and the development of plant genomics (Xiao et al., 2022).

Phenes are the building blocks of the plant phenotype, and phene states show the range of possible forms and functions for a given phene (York et al., 2013). The genotypic variance in many of the phenes is significant (Lynch et al., 2014). Breeders often largely miss crop processes, while crop physiologists frequently fail to understand the importance of varieties and cultivars on crop growth and development (Hay & Porter 2006).

To maintain long-term food security, crop resilience to abiotic stresses and novel pests brought on by climate change must be genetically improved (Tardieu et al., 2017). Invaluable options for generating novel alleles and selecting natural sources of genetic variation for crop improvement are provided by the growing use of gene editing and the ongoing exploitation of natural genetic diversity (Tester & Langridge, 2010). This requires analysis of hundreds of lines grown under diverse environmental scenarios. While breakthroughs in DNA marker assays and sequencing technology have allowed genotyping to attain this throughput at a fairly affordable cost (Cobb et al., 2013), similar advancements are urgently required to provide high-throughput and meaningful phenotypic information (Fiorani & Schurr, 2013).

The complex interaction of genetic information (G) with the multifactorial, abiotic, and biotic environment (E), as well as, in the case of crop plants, an agricultural management regime (M) that modifies the environment in a defined way, such as the application of fertilisers, irrigation, and pest and weed control, determines the plant phenotype. The transcription into RNA (transcriptome) and translation into proteins (proteome), which determine the different cellular metabolites (metabolome), are the fundamental processes in the expression of the information encoded in the genome. The interaction of structural elements, regulatory networks, flow through metabolic pathways, and enzymatic activity determines an organism's physiology. The actual phenotype is mostly the product of the integration of environmental elements and crop management into suitable cellular reactions at the level of physiology. In order to integrate the different levels of regulation involved in the expression of genetic information, including post-transcriptional, post-translational, and epigenetic mechanisms, the plant physiology serves as an essential link between the genome and the phenotype. The physiology can be thought of as the internal, biochemical phenotype that connects the genotype and the exterior phenotype. The ability to trace causal relationships between genotypes (G), environmental factors (E, M), and phenotypes will thus be made possible by integrating a high-resolution physiological phenotyping technique with wet chemistry investigations at the cellular level into phenomics. This will help to dissect the genetics of complex quantitative traits, particularly those related to yield, pathogen resistance, and abiotic stress tolerance, into defined physiological traits that can be more easily measured (Großkinsky et al., 2015).

Understanding biological determinants and how a plant's genetic makeup interacts with its environment requires a thorough study and characterization of the quantitative phenotypic variation that a plant genotype can occupy, which corresponds to the theoretical entity of the plant phenome. In order to shed light on the intricate, multifaceted character of phenotypes, phenomics—the comprehensive study of phenotypes—was developed (Houle et al., 2010). Quantitative genetics still has a number of systemic limitations due to the inaccuracy of phenotyping across a variety of contexts and geographical and time-based dimensions (Bazakos et al., 2017).

Due to the diversity of the genetic components, complex interactions between the genome, environment, and management have an impact on plants and determine phenotypic plasticity. Whereas great advances have been made in the cost-efficient and high-throughput analyses of genetic information and non-invasive phenotyping, the large-scale analyses of the underlying physiological mechanisms lag left behind. It is important to take into account these different scales of dynamic physiological responses, and genotyping and external phenotyping should be connected to physiology at the cellular and tissue level. A high-dimensional physiological phenotyping across scales integrates the precise characterization of the internal phenotype into high-throughput phenotyping of whole plants and canopies. By this means, complex traits can be broken down into individual components of physiological traits (Großkinsky et al., 2015).

Dimensionality, resolution, and throughput are the three factors that define phenotyping systems, and these systems should satisfy a number of important criteria, such as reproducibility, high throughput, adaptability to environmental conditions, noninvasiveness, and integration of phenotypes at various scales. One of the most important factors is reproducibility, which is also a result of homogeneity in growth circumstances and measurement. Nongenetic variation brought on by uncontrolled environmental disturbances should be avoided or reduced. For large-scale investigations using nonautomated phenotyping methods, throughput is a limiting issue, but it is also a limiting factor when assessing features that require frequent and effective screening (Dhondt et al., 2013). Continuous and repeated measurements at various scales, from a single cell to a whole root or plant, are possible with nondestructive phenotyping throughout a plant's life cycle. Beyond imaging in the visible spectrum, image acquisition methods like X-ray, MRI, fluorescence, near-infrared, infrared (thermal), multi/hyperspectral, and infrared (NIR) technologies can offer a vast quantity of phenotyping data on individuals over the course of their lives. In high-throughput trials, these data enable detailed analysis and integration of phenotypes at various scales, however calibration, signal processing, and picture analysis may still pose limitations. These phenotyping tools are also valuable for destructive "-omics" studies, including genomics, transcriptomics, proteomics, and metabolomics studies, which are advantageously performed on samples from individuals deeply characterized under precise conditions (Cubillos et al., 2014).

Donald (1968) suggested the ideatype, or trait-based breeding, as a strategy to combine characteristics that would individually help increase crop yields. He pointed out a problem with "deficit elimination" and "selection for yield" strategies in that they don't try to explain how higher yield is produced. As an alternative, he suggested looking at variables separately to understand how they affect yield before combining such yield-improving traits through traditional breeding. Crop breeding programs commonly combine traits, especially in the pyramiding of traits associated with disease resistance. Several crops' yield increases have benefited greatly from this strategy. The trait-based approach inherent in the concept of ideotype breeding to not only consider traits of interest in isolation, but also to consider relationships among traits. As an example, compensation among plant organs can lead to tradeoffs, such as increasing head numbers being associated with fewer, smaller kernels in barley. An overlooked part of ideotype breeding is the integration of characteristics, which impacts how the entire plant performs (York et al. 2013).

2. Plant phenes with a focus on roots

Numerous root anatomical phenes have an impact on the uptake of water from drying soil and may be useful in developing better drought-tolerant crops. Anatomical traits that reduce the root cortex's metabolic burden (or "cortical burden") enhance soil exploration and, consequently, the ability to extract water from a drying soil. Rhizosheaths can protect the immature root tissue's water status. Root penetration of hard, dry soil is improved by root hairs and greater diameter root tips. A large amount of these phenes exhibit significant genotypic variance. A promising but underutilised and undeveloped source of crop breeding goals is root anatomical phenes (Lynch et al., 2014).

In terrestrial ecosystems, inadequate water and nutrient availability is the main factor limiting plant development. Plant fitness and agricultural productivity thus depend significantly on the ability of plant roots to acquire soil nutrients. A group of interconnected characteristics (or phenes), make up plant root systems. Root phenes can be categorised according to how they influence the exploration or exploitation of resources, how they influence the acquisition of resources, and whether they have a neutral or influencing metabolic effect. These divisions establish the foraging strategies or metabolic economics that will be used by one phene to engage with another phene. Phenes that influence one another through foraging mechanisms are likely to operate within a phene module, a group of interacting phenes, that may be co-selected. Examples of root phene interactions are: 1) root hair length × root hair density, 2) lateral branching × root cortical aerenchyma (RCA), 3) adventitious root number × adventitious root respiration and basal root growth angle (BRGA), 4) nodal root number × RCA, and (5) BRGA × root hair length and density. By using simulation modelling and near-isophenic lines, which enable the study of particular phenes and phene combinations within a shared phenotypic background, advancement in the study of phenes and phene interactions will be enabled. It will be necessary to explore novel fields of research to better understand how phenotypic integration affects plant function in various contexts in order to have a comprehensive understand of the phenome at the organismal level. In order to develop crops with improved stress tolerance and less reliance on intensive input use, a better knowledge of how root phenes interact to effect soil resource acquisition will be a crucial tool (York et al., 2013).

Numerous crops have been found to exhibit genetic variation in root characteristics, which has functional implications for water uptake and improved yields in water-stressed environments. Differences in anatomical traits can lower the metabolic cost of soil investigation (Burton et al., 2013) and root architectural phenes can optimise soil exploration in time and space (Henry et al., 2011). In order to maintain soil exploration and water capture and to increase the economy of water use by shoots by increasing water use efficiency and lowering overall water demand, anatomical phenes that diminish the hydraulic conductivity of root systems under drought may be crucial (Lynch et al., 2014).

Roots that lower the metabolic cost of soil exploration are attractive and required by global agriculture (Lynch, 2015). Although rooting depth is frequently suggested as a selection criterion (Wasson et al. 2012), rooting depth is not itself a phene state that is subject to direct selection in a breeding programme (York et al. 2013). Instead, rooting depth can be impacted by a variety of root phenes that are under distinct genetic regulation. It is possible that the fitness value of root phene states is not constant and significant genotypic variation reported for many root phenes. There are various benefits to trait-based selection, including: 1) Useful traits may be present in germplasm that has poor yield potential, is not acclimated to the selection environment, or has other barriers to direct deployment in a breeding programme. 2) Traits are generally more durable and stable than yield itself. As a result, trait-based selection programmes can make use of a much wider variety of genetic variation than is typically found in the subset of germplasm that has a high yield potential in a particular environment. Additionally, the impact of traits on performance may involve significant environmental interactions. Trait selection makes it easier to design crops that are adapted to particular production situations by explicitly taking these interactions into account. 4) The impact of a trait on performance may have significant interactions with the expression of other characteristics. By maximising positive trait synergies and minimising negative trait interactions, trait selection enables the combining of traits in the best possible configurations (Lynch, 2015).

In many regions of the world, soil compaction negatively impacts agricultural productivity. Strong or compacted soil layers limit root exploration and availability to water and nutrients, which speeds up the onset of stress. Mechanical impedance and water stress together typically limit root development in drying soil. By enhancing the use of water in deep soil strata, deep rooting is vital for drought adaptation (Lynch, 2013), however the presence of hard or compacted soil layers frequently prevents the exploitation of deep water and nutrients. This means that increasing yield of crops in compacted soils requires deep roots that can penetrate hard soil. In addition to being related with lower cell sizes in the distal cortical region, root anatomical phenes were found to be more accurate predictors of root penetrability than root diameter alone. Smaller outer cortical area cells are essential for preventing the root from ovalizing and lowering the possibility of local buckling and collapse during penetration, which increases the penetration of hard layers into the root. Root tensile strength was shown to be more accurately predicted by stele diameter than by root diameter. Root bend strength was better predicted by cortical thickness, cortical cell count, cortical cell wall area, and distal cortical cell size than by root diameter. Root penetrability of high-strength layers and root biomechanical characteristics are significantly predicted by root anatomical phenes (Chimungu et al., 2015). According to Jin et al. (2013), phenes that decrease the chance of root buckling while penetrating hard soils are generally associated with the ability of roots to penetrate hard soils. Anatomical phenes, such as high root cortical aerenchyma, small living cortical area, decreased cortical cell file number, and large cortical cells, have been linked to effective soil resource acquisition under abiotic pressures (Zhu et al., 2010).

3. High-throughput analysis and automated phenotyping platforms

Genetic variation for root traits required for breeding has been found in several crops using both seedling and mature plant phenotyping platforms (Tracy et al., 2020), including maize (Zea mays L.; Gao & Lynch, 2016), soybean (Glycine max; Prince et al., 2018), common bean (Strock et al., 2019), and cowpea (Burridge et al., 2017). Quantitative data on genotype-environment correlations holds the key to solving some of the greatest challenges facing the globe. Plant phenotyping, the evaluation of complex plant properties including growth, development, tolerance, resistance, etc., has turned into a major bottleneck. The plant research community must precisely assess progressively more plants and plant characteristics in order to support and speed progress in breeding for novel traits (Niederbacher et al., 2015). As a result, high-throughput analysis techniques have become state-of-the-art in the biological sciences over the past few years (Hartmann et al., 2011). One of the most recent innovations to enable non-destructive screening of plants over certain time intervals using image capturing techniques is an automated greenhouse system for high-throughput plant phenotyping (Hartmann et al., 2011).

Fig. 1. 1680 plants can be grown under controlled conditions of soil water status and temperature, imaged, and evaluated for transpiration rate on the phenotyping platform (PhenoArch). Sensors monitor air and leaf temperature, light, relative humidity, and transpiration (Cabrera-Bosquet et al., 2016).

The study of plant phenomics experiences a conceptual difficulty. Researchers have thus far concentrated on using and/or designing novel sensors and imaging methods. Methodological advancements in data collection, handling, and processing, however, are becoming more and more essential. Indeed, the challenges of translating sensor information into knowledge have been grossly underestimated during the first years of plant phenomics research. In order to meet this issue, it is necessary to take into account the close relationship that exists between environmental factors and plant structure, functions, and metabolism. As a result, all phenotyping phases, from data collecting to meta-analyses, must incorporate environmental characterisation. In order to cope with the studied plant characteristics, it also necessitates the employment of dynamic and statistical models that enable multi-scale analyses across trials and platforms. The most recent advances in information technology must be employed to face the big-data challenge associated with multi-image processing, of meta-analysis of heterogeneous data and of the deployment of phenomics beyond the strict world of research. It won't be possible to monitor all temporal and organisational scales in all environments due to obvious financial constraints, but we believe that the rapid advancement of modelling and information systems will enable the identification of suitable equipment, methods, and meta-analyses allowing resource optimisation. (Tardieu et al., 2017).

Depending on the overall design, phenotyping systems can be categorised as either plant-to-sensor (Golzarian et al., 2011) or sensor-to-plant (Granier et al., 2006), depending on whether the plants are transported to an imaging station or remain in a fixed position during a measurement routine. The majority of these platforms are particularly ideal for controlled water-limitation experiments that automatically irrigate each pot to a predetermined goal weight while using gravimetric methods to assess daily evapotranspiration (Berger et al., 2010). The most commonly used method for evaluating growth and rosette geometry time courses is 2D RGB (red, green, blue) imaging followed by image preprocessing and segmentation for the extraction of projected shoot area and geometric parameters (Mittler & Blumwald, 2010).

Research on remote sensing of vegetation is where imaging spectroscopy for plant phenotyping first emerged. Multispectral and hyperspectral cameras that can scan wavebands of interest at high resolutions, particularly near the peak of green reflectance at 550 nm and the water absorption bands in the near-infrared (NIR) to mid-infrared region, have made it possible to perform spectral measurements for a larger portion of the electromagnetic spectrum (Trachsel et al., 2011). Strong water-absorbing bands at 970 nm, 1,200 nm, 1,450 nm, 1,930 nm, and 2,500 nm are present in this region (Fiorani & Schurr, 2013). To assess the water status of leaves, thermal imaging measurements of leaf and canopy temperature (3-14 m spectral range) have been used in both lab and field settings (Munns et al., 2010). Currently, cereal breeding programmes use canopy temperature depression as a selection characteristic for drought resilience in dry areas. Canopy temperature depression is the difference in temperature between the canopy and the surrounding air (Fiorani & Schurr, 2013).

Fluorescence of chlorophyll is frequently used at both laboratory and field scales. It provides a quick technique to check the health of the photosystem II in vivo. Recent researches suggested several ways to use chlorophyll fluorescence to detect early stress responses to biotic and abiotic variables before a growth slowdown may be seen (Fiorani & Schurr, 2013). In recent works describing automated high-throughput analysis to study plant stress responses uses platforms PHENOPSIS, WIWAM, PHENOSCOPE, LemnaTec, LemnaTec, PlantScreen (Humplík et al., 2015).

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CHAPTER 10

FACTORS AFFECTING THE POST-HARVEST QUALITY OF CUT FLOWERS

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1. INTRODUCTION

The vase life of cut flowers is defined as the time from when the stems are placed in the vase solution until they lose their visual quality (Halevy and Mayak, 1981). It is desirable that cut flowers have a long vase life due to affecting consumer satisfaction and demand for flowers.

Cut flowers produced in the world are lost due to various reasons from reaching the producer to the consumer. Due to the inability to provide suitable conditions before and after the harvest, some damages occur in the quality of the stem, leaves and flower organs. In addition to these, factors such as the negative effects of diseases and pests, inability to establish a cold chain and unsuitable environmental conditions, affect the quality and product losses in cut flowers. Although it varies according to species and varieties, the loss rate is around 25% in the world, while it is estimated to be around 30-50% in production for domestic consumption in our country.

Pre-harvest, during-harvest and post-harvest factors affect the vase life of cut flowers (Mengüç, 1991; Fanourakis et al., 2012; Kazaz, 2015). The preservation of the quality of the flowers produced in the most suitable conditions and their delivery to the end consumer depend on the post-harvest factors (Çelikel, 2020). The factors affecting the vase life of cut flowers are listed below.

2. PRE-HARVEST FACTORS

Genetic structure and growing conditions in cut flowers are the main pre-harvest factors affecting vase life. Knowing the optimum environmental conditions and genotype interaction is important in maximizing the post-harvest endurance of flowers (Van Meeteren et al., 2005; Fanourakis et al., 2012). It has been reported that the environmental conditions in which cut flowers are grown and cultural processes (such as irrigation, fertilization, disease and pest control) prolong the vase life due to their positive effects on increasing dry matter accumulation and stem thickness (Orçun and Erdem 1973; Uzun et al., 1983).

Senescence is defined as the final stage of the plants that results in the termination of the cell, tissue, organs or life cycle (Ok Lim et al., 2007; Liu et al., 2008; Gregersen et al., 2013; Gully et al., 2015). It has been reported that unsuitable environmental conditions (temperature, drought, air humidity, nutrient deficiency, light, disease and pathogen factors) and genetic structure affect senescence on plants (Christiansen and Gregersen, 2014; Penfold and Buchanan-Wollaston, 2014).

3. FACTORS DURING HARVEST

Harvest time affects the post-harvest endurance of cut flowers. Therefore, it is important to determine the harvest time, where and how to harvest. The stage at which the flower will be harvested affects first on the flower and then the opening of the flower (Moe, 1975; Marissen and Benninga, 2001). The harvesting stages of flowers vary according to species and cultivars (Mortensen and Fjeld, 1998; Sonneveld et al., 1999; Särkkä and Rita, 2002; Gorbe, 2009). The most appropriate harvest period for cut flowers has been defined as the commercial harvest phase (Ferreira and de Swardt, 1981; Slootweg and Van Meeteren, 1991; Mortensen and Gislerød, 1999, 2011; Khoshgoftarmanesh et al., 2008; Borda et al., 2011; Victoria et al., 2012). Harvest time and cutting surface of flowers affect on vase life (Crow, 1970).

It has been reported that the most suitable time for harvesting is early in the morning or in the evening chill (Anonymous, 2002). Harvesting at noon causes excessive water loss and wilting in flowers. Because the temperature and relative humidity are low in the early morning and the water content of the plant is high, the morning harvest is more beneficial than the noon harvest (Van Doorn and Suiro, 1996; Wilkins, 1998; Dole and Schenelle, 2002). In open field cultivation, the most suitable harvest time is harvesting in the early morning hours after the drying the dew on the plants (Salinger JP, 1987). Determining the harvest phase of cut flowers varies according to species and variety, time and season, marketing style, market distance, consumer demands and environmental conditions.

Flowers to be sent to long distances are harvested earlier than flowers to be sent to close distances (Redman et al., 2002). The flowers to be sent to distant markets or stored for later evaluation, are harvested at the firm bud stage and then opened with the help of solutions that promote flowering. It is recommended to cut the herbaceous flowers cut at an angle. Cutting tools used during harvest must be sharp and disinfected daily. To avoid the risk of disease or pest contamination, harvested flowers should not be placed directly on the soil (Gast, 1997; Wilkins, 1998; Dole and Schnelle, 2002).

During harvest, air enters the vascular bundles in the cut flower stems and causes air embolism In order to facilitate the water intake of the flowers before water extraction, 1-2.5 cm of the stem from the bottom is cut at an angle (Broun, 1981; Dole and Schnele, 2002). The flower stems are cut underwater to prevent air from entering the stems, but this practice is often impractical (Rogers, 1973).

4. POST-HARVEST FACTORS

Halevy and Mayak (1979) reported that the vase life of cut flowers was affected by 2/3 of preharvest factors and 1/3 of postharvest factors. Although the vase life is a factor that can be easily determined, vase life measurements of the same flower at different times under similar conditions may show different results (Fanourakis, 2013). After harvesting, cut flowers undergoes different processes such as sorting, leaf plucking, bunching, re-cutting, water extraction, treatment with flower preservative solutions, packaging/wrapping, pre-cooling, storage and transportation. Factors such as blocking of vascular bundles, ethylene, leaf yellowing, geotropism, storage and transportation conditions affect the shortening of the vase life of flowers. The harvested flowers are classified according to variety standards and stem length. It is desired that the classified flowers should have smooth and upright stems, and on the leaves there should not be disease, pests and mechanically damaged, (Kazaz et al., 2003).

It is important to pluck the leaves of the flowers placed in the vase, which is above the water level in order not to clog the vascular bundles due to bacterial and fungal growth in the vase water (Broun, 1981; Gast, 1997).

In order to regain the water lost after harvest, cut flowers are kept in water with a low pH (3.5-5) at an ambient temperature of 4 ^{0}C to regain turgor (Broun, 1981; Wilkins 1998).

Cut flower preservative solutions are defined as preparations that is used to increase water intake and generally contains water, sugar, germicide, acid regulator and prevents/slows down undesirable conditions such as ethylene damage, aging, leaf yellowing, microbial growth (Vaughan, 1988; Gast, 1997).

4.1. Blocking of Vascular Bundles

From the moment they are harvested, cut flowers get their hydration from the vase water they are placed in. Blockages in the transmission bundles cause the flowers to not receive enough hydration. The vascular bundles of flowers are blocked due to causes such as microbial activity (bacteria, fungi, etc.), physiological clogging (biochemical substances) and air embolism (Woltering, 1987; Put and Jansen, 1989; Kazaz et al., 2003).

Microorganisms such as bacteria and fungi can live in the vase water in which cut flowers are placed. Bacteria (*achromobacter, bacillus, micrococcus and pseudomonas*) that cause blockage of the conduction bundles cause blockage either directly or with the toxic substances they secrete. It has been reported that bacterial density is more effective than bacterial species in the obstruction of the conduction bundles (De Witte and Van Doorn, 1988).

Microbial blocking is seen only at the cut sites whereas physiological blocking starts from the tip of the flower stems and continues upwards. It is estimated that physiological blocking is caused by oxidation at the cut-off point whereas the blockage occurring in injured flowers is more likely to be caused by lignin formed as a result of tylosis. It is reported that the blocking of vascular bundles in the stem is caused by substances formed by degrade of pectins (Baktır, 1983).

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

4.2. Ethylene

Ethylene is a natural plant hormone synthesized in the plant, which is also effective in the growth and aging of plants. Controlling ethylene is important for maintaining the post-harvest quality of cut flowers. Ethylene releasing from plant or environmental sources shortens vase life by causing flower, leaf and petal fall, leaf yellowing, necrosis and aging. Ethylene also affects bud opening beside aging of petals and leaves. It has been reported that cut flower species have different levels of ethylene sensitivity. For this reason, ethylene inhibitors have an important place in the post-harvest applications of cut flowers (Çelikel, 2020). Ethylene sensitivity of some cut flower species is shown in Table 4.1.

Table 4.1 Sensitivity of some cut flower species to ethylene (Kazaz, 2015)

Sensitive species	Medium-level sensitive species	Non sensitive species
Alstroemeria	Cut rose	Chrysanthemum
Gypsophila	Narcissus	Iris
Carnation	Lisianthus	Gladiolus
Lilium	Orchid (Dendrobium)	Gerbera
Orchid (Phalaenopsis)	Waxflower	Strelitzia
Orkide (Cymbidium)	Cut sunflower	Tulipa
Sweet william	Larkspur	Anthurium
Gillyflower		Freesia
Limonium		Goldenrod

Preventing or reducing ethylene damage in cut flower species sensitive to ethylene; flowers should be harvested at the optimum development period, pre-cooling should be done after harvest, ventilation and air flow should be provided in product processing and storage areas, flowers should not be stored together with products that produce ethylene (fruits and vegetables), products that prevent the effect of ethylene should be used. Slowing down or stopping the formation of ethylene directly affects aging. Various chemicals are used to reduce ethylene formation (Kazaz et al., 2003). Some anti-ethylene products used in vase solutions and their doses are shown in Table 4.2.

Table 4.2 Some anti-ethylene products used in vase solutions

Product name	Application Dosage
Nano silver (NS)	0,1-5 ppm
Silver thiosulfate (STS)	1-3 ml/L
1-Methylcyclopropane (1-MCP)	2.5 nl-1 µl/L
Methoxyvinyl (MGV)	5-100 ppm
Aminoethoxyvinylglycine (AVG)	5-100 ppm
Aminooxyacetic acid (AOA)	0,5- 4 µM
Potassium manganate (KP)	0,5- 1 µM

4.3. Leaf Yellowing

Leaf yellowing is an important problem in some cut flower species such as solidago, lily, chrysanthemum, alstroemeria and gillyflower (Kazaz, 2015; Çelikel, 2020; Aydın et al., 2022). Leaf yellowing causes the decrease in the quality of flowers and the shortening of the vase life (Aydın et al., 2022). The effect of the products used against leaf yellowing varies according to the species and cultivars. Products such as gibberellins (GA_3), cytokinins (benzylaminopurine, zeatin) and thidiazuron are used as a spray or as a solution uptake from the stem (Philosoph-Hadas, 1996; Hassan et al., 2003; Çelikel, 2013). Leaf yellowing can be controlled by using of plant growth regulators at various concentrations (Thomas and Stoddart, 1980; Thimann, 1985). Cytokinins which are plant growth regulators, significantly delay or reverse leaf yellowing in some species have been reported by many researchers (Gvilli and Mayak, 1970; Staden, 1978; D'Hont et al., 1991; Hicklenton, 1991; Han, 1995). Auxin, cytokinin and gibberellins delay chlorophyll degradation, while ethylene and abscisic acid increase chlorophyll degradation (Nooden, 1988; D'Hont et al., 1991; Van Doom et al., 1992; Van Doom and Van Lieburg, 1993; Han, 1995). Light (1000 lux) delays aging in species which have leaf yellowing, and accelerates water loss by opening stomata in other species (Wilkins, 1998; Vijayakumar et al., 2021).

4.4. Geotropism

Geotropism is defined as the response of flowers to gravity during transportation and storage depending on placing them horizontally. In some cut flower species that are sensitive to geotropism (tulip, ranunculus, snapdragon, gerbera, gladiolus, lisianthus and gillyflower etc.) the spikes are bent upwards, causing negative geotropism and deterioration in quality. Therefore, cut flower species sensitive to geotropism should always be stored/transported vertically (Dole and Schnelle, 2002).

4.5. Storage and Transportation Conditions

The main function of storage is to reduce the metabolic activity thanks to low temperature and to ensure that the products last longer (Ağaoğlu et al., 1995). Low temperature is the most important factor in maintaining the quality of cut flowers during storage (Vandoorn and Witte, 1991).

After the storage of cut flowers, many problems (non-opening of buds, over-blowing of flowers, deterioration of petal color, leaf yellowing, development of disease, decrease in strength) can be encountered (Kazaz et al., 2003). It has been reported that the post-harvest life of cut flowers varies according to the type and variety of the plant (Uzun et al., 1983).

As the storage temperature decreases, respiration, metabolic activity, ethylene synthesis and disease development decrease. As a result of this, the post-harvest durability period increases. For this reason, many cut flower species and varieties are stored at 0 ^{0}C just above the freezing point. Tropical climate flowers (orchids and anthuriums) show cold damage at low temperatures (below 10 ^{o}C). Therefore, the storage or transportation of such flowers should be above 10 ^{o}C (Kazaz, 2015). The storage temperatures of some cut flower species are given in Table 4.3.

Table 4.3 Storage temperatures of some cut flower species (Kazaz, 2015)

Species	**Storage temperature (^{o}C)**	**Species**	**Storage temperature (^{o}C)**
Carnation	0-1	Gerbera	0-1
Chrysanthemum	0-1	Iris	0
Cut Rose	0-1	Solidago	0-1
Narcissus	0-1	Lily	2-4
Freesia	0-1	Gladiolus	2-5
Tulip	0-1	Gypsophila	0-1
Alstromeria	0-1	Anthurium	12.5-20
Lisianthus	0-1	Orchid	10-14

Cut flowers should be stored in a shaded place out of direct sunlight after harvesting. The light increases the color intensity in the blooming of the flowers that are harvested in the not fully opened form while in some species (lilium, alstromeria, gillyflower, chrysanthemum, limonium, miniature gladiolus, etc.) it prevents leaf yellowing (Wilkins, 1998; Anonymous, 2002).

During the storage of flowers, high humidity (%85-95) is desired. High humidity can cause water droplets to form on the flowers and leaves and the formation of fungal diseases (botrytis). Low humidity causes flowers to lose water and shorten their vase life (Dole and schenelle, 2002; Vijayakumar et al., 2021).

Air movement in the storage room should be kept low and flowers should not be exposed to direct air movement. Flower boxes should be placed on shelves in the warehouse, air flow should pass between and around the boxes. Shelves should be at least 5 cm high from the floor and should not prevent the passage of airflow. Insufficient and irregular air circulation causes rapid maturation and aging of the product, water loss and disease increases (Faragher et al., 2002).

4.6. Packaging/wrapping

Packaging is done to prevent mechanical damage that may occur during the storage and transportation of flowers. The packaging/wrapping materials, being perforated or containing air, prevent condensation and reduce the risk of botrytis. In order to reduce physical damage when packaging cut flowers, flat and long boxes that are not too deep should be preferred (Mrema, 2002).

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CHAPTER 11

MANAGEMENT OF POSTHARVEST DISEASES AND MAINTAINING OF FRUIT QUALITY WITH ANTAGONISTIC MICROORGANISMS

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1. Giriş

In the last few decades, interest and research towards finding alternative approaches to manage plant diseases without using chemical pesticides have increased. Factors such as sustainable agricultural production systems, organic farming, public reluctance towards genetically modified products have contributed significantly to this shift in focus. Regulatory agencies of governments have become more restrictive on materials and products used in agricultural production due to legitimate health and safety concerns as well as public pressure (Çavuşoğlu at al., 2020). The use of post-harvest fungicides has been particularly affected by this situation, which has had significant effects on the export and shipment of products to foreign markets. The European Union has taken a leading role in reducing the use of synthetic pesticides and residues allowed in products sold to consumers (Yılmaz et al., 2022).

Some alternatives to synthetic pesticides include microbial pesticides derived from microorganisms like bacteria, fungi, viruses, protozoa, and algae, phytopesticides such as essential oils and extracts from different parts of plants, biological control which is an alternative source for managing plant diseases, and botanical pesticides obtained naturally from plant-based products such as neem-based pesticides, pyrethrum, and eucalyptus oil. Nano-based pesticides are also being explored as an efficient alternative for pest and disease management in agriculture without harming natüre (Wang et al., 2022)

Large supermarket chains and wholesale fruit suppliers have established their own standards for the chemical residues allowed in harvested products and the materials used throughout the growing season. Concerns about mycotoxins and foodborne pathogens have also increased the need for alternative methods in plant disease management. Indeed, restrictions on preharvest fungicides have led to increased levels of postharvest pathogens and hidden infections at harvest (Sheng at al., 2023; Droby at al., 2019).

Finding alternative methods for managing post-harvest diseases is a process that includes legal requirements, testing under the ecological conditions where the products are grown, consumer acceptance, and commercial feasibility (Cavusoglu at al., 2021a). In recent years, a notable approach has been the use of microbial antagonists applied to harvested products as biocontrol agents to manage various post-harvest pathogens. Bacterial and yeast antagonists have been isolated from fruit surfaces and reported to exhibit high efficacy in laboratory and pilot tests conducted under semi-commercial conditions (Carmona-Hernandez at al., 2019; Droby at al., 2019).

This section provides a general overview of the use of microbial antagonists as postharvest biocontrol agents. It covers various aspects related to the procedures used to identify potential antagonists, mechanisms contributing to biocontrol activity, parameters associated with the commercialization and use of post-harvest biocontrol products, and how the use of antagonists can be integrated with other alternative disease management methods.

2. Discovery, Isolation, Visualization and Identification of Biocontrol Agents and Antagonists

The use of fungal and bacterial species to modify or preserve food has been an integral part of human civilization. Translating this concept into a scientific approach to managing post-harvest rot may make more sense than forcing it. In terms of postharvest on biocontrol, it is presumed that microbial strains antagonistic to decay fungi are existing on the skin of harvested cereals as well as on the surface of fruits and vegetables. By selecting these species and treating them in large amounts on the surface of harvested crops, the shelf life of the products can be prolonged without the use of such synthetic chemicals (Ye at al., 2021; Wang at al., 2021; Droby at al., 2019).

Isolation of biocontrol agents is a very important step in the process of identifying new pest management agents. The isolation process consists of selective growing of microorganisms from various resources such as soil, plant roots, leaves and rhizosphere. To obtain pure cultures of microorganisms that exhibit antagonistic activity against the host pathogen, selective medium and techniques such as serial dilution cultivation, broadcast cultivation or line cultivation are employed. These methods are widely utilized to isolate diverse biocontrol agents such as bacteria, fungi and viruses (Xiao at al., 2021; Droby at al., 2019)

Serial dilution culture is a method that is used to dilute a stock solution of bacteria or other microorganisms to a concentrate that is more easily counted. The diluted sample is then spread on an agar plate and Incubated. Plating is a technique that is used to isolate the individual bacterial colonies on an agar plate. A small amount of the sample is spread on the agar surfaces by using a sterile spreader. Plate inoculation is a technique that is used to isolate individual bacterial colonies on an agar plate. A small amount of sample is spread on the agar surface by using a sterile ring. Serial dilution, plate inoculation and plate cultivation are used in order to obtain pure cultures of microorganisms that exhibit antagonistic activity against a target pathogen. These methods are commonly used for isolating various biocontrol agents such as bacteria, fungi and viruses. Selective cultivation of microorganisms from a variety of sources such as soil, plant roots, leaves, and rhizosphere is performed by using selective media and techniques such as serial dilution cultivation, plate cultivation, and plate seeding (Haney at al., 2021)

The screening is used to evaluate the effectiveness of the isolated biocontrol agents towards the host-pathogen. Several screening methods have been utilized to evaluate the effectiveness of the isolated biocontrol agents, such as dual culture tests, plucked leaf tests, and greenhouse tests. The dual culture test consists of co-culture of the biocontrol agents on the target pathogen (Sinno at al., 2021).

3. Mechanisms of Antagonistic Microorganisms

Antagonistic microorganisms have been detected as potential biological control agents to prevent postharvest decay of fruits and vegetables. These microorganisms act through various mechanisms to inhibit the growth and survival of pathogenic microorganisms, thereby reducing postharvest losses (Konsue at al., 2020).

Mechanisms of Action of Antagonistic Microorganisms:

1. Competition for Nutrients Antagonistic microorganisms can compete with pathogens for nutrients, depriving them of resources necessary for growth and survival. Competition for nutrients and space is a crucial mechanism used by antagonists because both pathogen and antagonist compete for the same resources, leading to niche overlap. Yeasts and bacteria have an advantage in this competition due to their ability to multiply and colonize (Droby et al., 1989; Spadaro et al., 2010). They can also form an extracellular polysaccharide capsule facilitating adhesion to the fruit surface. Biofilm formation inside wounds, involving the encapsulation of microorganisms in a hydrated matrix, promotes competitive interactions (Lutz et al., 2013).

Biofilm-forming yeasts showed activity against Penicillium expansum Link in apples. However, it has been observed that only yeast cells collected from the biofilm phase can effectively settle on the inner surface of the wounds (Ianiri et al., 2013). Competition for nutrients, especially iron, becomes crucial in the context of fruit sores. Iron is essential for fungal growth and pathogenesis, and the breakdown of iron by non-pathogenic microorganisms can be used in postharvest biocontrol systems. When fungi experience iron starvation, their catalase activity decreases, and their resistance to reactive oxygen species (ROS) decreases. Rhodotorulic acid, a siderophore produced by Rhodotorula glutinis, has increased blue mold control in apples (Calvente et al., 1999). Metschnikowia pulcherrima and M. fructicola are capable of producing the red pigment pulcherrimin, which is derived from pulcherriminic acid and ferric ions. This pigment manages apple diseases (Spadaro and Droby, 2016).

2. Antibiosis: Antagonistic microorganisms can produce various antimicrobial compounds, such as antibiotics, enzymes, and volatile organic compounds, that inhibit the growth and survival of pathogenic microorganisms. Antibiosis refers to the process in which a microorganism is inhibited or destroyed by diffusible or volatile antibiotics produced by another microorganism. In certain circumstances, when substrate availability decreases, some microorganisms initiate the production of antibiotics to prevent other microorganisms from utilizing the remaining substrate. Bacteriocins, which are antibacterial proteins produced by bacteria, function by inducing the formation of pores in the membrane of target cells, leading to a depletion of transmembrane potential and subsequent leakage of cell material. Bacillus subtilis, an antagonist microorganism, is known to produce several antimicrobial cyclic lipopeptides such as iturins, fengycins, and surfactins (Ongena and Jacques, 2008; Yánez-Mendizábal et al., 2012). Another example is Aureobasidium pullulans, which can produce aurebacidin, a cyclic depsipeptide with antifungal and antibiotic properties. When considering antibiotic-producing microorganisms in food products,

caution should be exercised regarding potential risks, such as the development of resistance to these compounds among fruit microorganisms and the potential transfer of resistance to human pathogens (Droby at al., 2019).

3. Induction of Host Defense Mechanisms: Antagonistic microorganisms could stimulate the host plant to activite defense mechanisms such as phytoalexins and pathogenesis-related proteins that inhibit the growth and survival of pathogenic microorganisms.
4. Biofilm Formation: Antagonistic microorganisms can form biofilms on the surface of fruit and vegetables, thereby creating a physical barrier that prevents the attachment and colonization of pathogenic microorganisms (Cavusoglu at al., 2021). Some antagonists may react with host tissue to promote cicatrisation and lignification of cell walls. Biocontrol agents may react with injured tissue to induce β-1,3-glucanase, chitinase and peroxidase activity, phytoalexin accumulation and the formation of structural barriers. Candida oleophila application induced phytoalexin production, ethylene autosynthesis and deposition of chitinase and β-1,3-glucanase enzymes in grapefruit (Droby et al., 2002). Cryptococcus laurentii was treated to jujube, β-1,3-glucanase activity enhanced and Glu-1 gene expression was highly increased in grapefruit (Droby et al., 2002). All results related to the induction of resistance after antagonist application are consistent. Nevertheless, direct proof of the ability of the substances induced to hinder pathogen infection and development has not been established. The indirect antagonism expresses the resistance induction by useful microorganisms in fruit tissue. These antagonists can interact with the host tissue, leading to increased cicatrisation (healing) and lignification (strengthening) of cell walls. Biocontrol agents, when in contact with wounded tissue, can induce the activity of enzymes such as β-1,3-glucanase, chitinase, and peroxidase, as well as promote the accumulation of phytoalexins (defense compounds) and the formation of structural barriers. For instance, the application of Candida oleophila on grapefruit can induce the production of phytoalexins, ethylene biosynthesis, and the accumulation of chitinase and β-1,3-glucanase enzymes (Droby et al.,

2002). Likewise, when Cryptococcus laurentii is applied to jujube fruit, it leads to increased β-1,3-glucanase activity and highly induced expression of the Glu-1 gene involved in postharvest defense reactions towards pathogens (Tian et al., 2007). However, it is critical to note that the results on the induction of resistance after treatment with antagonists are correlative and direct evidence on the ability of induced substances to prevent infection and pathogen growth is still incomplete (Droby at al., 2019).

4. Use of Antagonistic Microorganisms and Shortcomings of Postharvest Biocontrol

Antagonistic microorganisms have been investigated extensively recently as postharvest practices. These microorganisms are usually treated on the skin of fruits and vegetables as biocontrol agents, either alone or in combination with other applications such as hot water or fungicides. The effectiveness of postharvest applications with antagonistic microorganisms relies on several factors, such as the type of microorganism utilized, the potency of the application, and the duration of the application. Additionally, postharvest storage condition such as temperature, humidity, and storage time may also influence the effectiveness of the application (Droby at al., 2019).

Many studies have demonstrated the efficacy of postharvest treatments with antagonistic microorganisms to manage postharvest spoilage of fruits and vegetables. For example, Bacillus subtilis is effective against postharvest rot of apples and strawberries. Similarly, *Pseudomonas fluorescens* is effective on citrus postharvest rot (Wang et al., 2021; Dwiastuti at al., 2021). Overall, the use of antagonistic microorganisms as postharvest treatments can potentially reduce postharvest losses and improve the quality and safety of fruits and vegetables.

Despite several reports underlining the potential and commercial feasibility of antagonists, the product has not yet achieved broad use in postharvest disease control. several products were originally introduced but later withdrawn, while many others have succeeded only in restricted niche markets. The absence of this widespread adoption can be ascribed to various factors such as inconsistent performance, restricted industry acceptance, higher costs as compared to synthetic fungicides, regulatory hurdles and formulation issues (Droby et al., 2016).

The field of postharvest biocontrol has developed in a sophisticated way as new strategies to tackle these difficulties have been explored. Encompassing applications in packing houses, storage, transportation and other aspects of the supply chain, the post-harvest environment is now recognized as a system that requires a broad and holistic approach to address a wide range of issues. Droby et al., (2009) comprehensively analyzed the main barriers to the commercialization of postharvest biocontrol products. One of the biggest challenges is the need for consistent and reliable performance under various conditions, including different packaging lines, inoculum levels and the use of other technologies to maintain quality. Costs associated with product development, commercialization as well as industry acceptance are also factors limiting the adoption of alternative technologies.

Although there is a wealth of information on the use of biocontrol agents in controlling postharvest diseases, none of these approaches have been implemented on a large scale. Bridging the gap between research and industry is crucial to enable the commercial success of these alternatives. Some registered postharvest biocontrol products have been developed through collaborations between researchers and commercial companies (Droby et al., 2016). However, achieving commercial success, measured by acceptance and widespread use, has remained elusive. The efficacy of these products must be comparable to that of chemical fungicides, which typically exhibit disease control rates of 98-100%. Biological control products often fall short of this level of effectiveness when used as standalone treatments. Therefore, new ideas and approaches are needed to realize the commercial success of biological control in postharvest disease management and to implement it on a larger scale.

5. Enhancing biocontrol efficacy

The use of biocontrol agents for postharvest disease control often faces limitations due to the influence of various factors that are challenging to control. As mentioned earlier, these factors encompass temperature, relative humidity (RH), host physiology, existing infections, and preharvest treatment history. One notable limitation is that biocontrol agents generally do not provide sufficient control of established infections. Consequently, research efforts have focused on enhancing their efficacy by employing a combination of two or more postharvest treatments in a cascade, similar to the hurdle technology strategy employed in the food industry.

Combination with physical treatments: The integration of microbial antagonists with heat treatments, such as hot air and hot water treatments, has been one of the most successful and extensively studied approaches in combining physical treatments with biocontrol agents for postharvest disease control. Hot air and hot water treatments are relatively simple and practical techniques that can be readily implemented in packinghouses to reduce the incidence of postharvest diseases (Zhang at al., 2007).

These heat treatments involve subjecting the harvested fruit or vegetables to elevated temperatures for a specific duration. The heat serves multiple purposes: it directly kills or inhibits the growth of pathogens, it can induce stress responses in the produce that enhance their natural defense mechanisms, and it creates an inhospitable environment for pathogen survival and growth. By combining microbial antagonists integrated with heat treatments, biocontrol agents may also act in a synergistic manner through the use of heat to provide improved disease control (Conway at al., 2004; Zhang at al., 2017).

Combination with chemical products: The interaction of biocontrol agents with materials that are generally regarded as safe (GRAS), such as natural products and resistance inducers, has been a subject of investigation in postharvest disease control. GRAS such as salt additives have demonstrated their high potential to increase the effectiveness of microbial antagonists in the control of postharvest decay (Sharma at al., 2009).

Several salt additives such as calcium chloride, calcium propionate, sodium carbonate, sodium bicarbonate, potassium metabisulfite, and ammonium molybdate have been examined for their abilities to improve the performances of the microbial antagonists. The effectiveness of the interaction has been shown to rely on factors such as the concentrations of the antagonist, the concentration of the salt additives, their inter-compatibility, and the time and duration of the application (Sharma at al., 2009).

Carbonic acid salts such as sodium carbonate and sodium bicarbonate have been assessed as suitable targets for the incorporation of biocontrol agents for postharvest disease control, particularly in citrus. These are found to possess fungistatic properties, i.e. inhibit fungal growth, and are comparatively inexpensive, easily available and have a minimal risk of causing damage to the fruit. They can be used in combination with biocontrol agents to enable an integrate address to postharvest disease management in citrus (Porat at al., 2002; De Costa at al., 2012).

Research studies have demonstrated that combination of microbial antagonists together with carbonic acid salts can enhance disease resistance and prolong the shelf life of citrus fruit. These salts can enhances the capability of antagonists to hinder pathogen overgrowth, prevent spore germination and decrease the incidence of decay (citrus Porat at al., 2002; Çavuşoğlu at al., 2021b).

6. Conclusion

Concern over pesticide residues in fresh fruits and vegetables has resulted in an increased demand for innovative management approaches that minimize or eradicate the use of synthetic chemical fungicides. As major post-harvest fungicides are removed from the market because of regulatory limitations or high reregistration costs, the practical need for instruments to minimize postharvest losses is becoming more obvious. Biocontrol products, notably yeast antagonists, are expected to achieve momentum and get broadly accepted as part of an integrated approach to managing postharvest diseases (Droby at al., 2019).

On the other hand, many challenges need to be overcome in order to get commercially successful postharvest biocontrol products. These challenges include: a) Improving and increasing the efficacy of biocontrol agents under commercial conditions. It is crucial to optimize the performance of yeast antagonists to ensure consistent and reliable disease control in real-world environments. b) Develop cost-effective, high-quality fermentation and formulation methods. Efficient production processes and formulation techniques are essential to commercially make biocontrol products economically viable. c) Maintain cell viability and biocontrol efficacy in the formulated product. Biocontrol formulations' stability and shelf life are crucial to ensure their efficacy during storage and application. d) Identification of yeast antagonists with a broad spectrum of activity against different pathogens in various products. It is essential to find biocontrol agents that can act effectively against multiple pathogens and provide a broader range of applications in different products. e) Establishing effective marketing outlets, preferably through multinational companies. Successful commercialization of biocontrol products requires strong marketing and distribution networks to reach a wide range of growers and consumers. f) Developing a fundamental understanding of how biocontrol systems operate and how environmental factors influence the interactions between the host, pathogen, and biocontrol agent. A comprehensive understanding of these interactions will help optimize biocontrol strategies and improve their performance.

Addressing these challenges through ongoing research and collaboration between academia, industry, and regulatory agencies will contribute to the development and adoption of effective postharvest biocontrol products. With the increasing demand for sustainable and residue-free agricultural practices, the use of biocontrol agents is expected to play a crucial role in future postharvest disease management strategies.

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