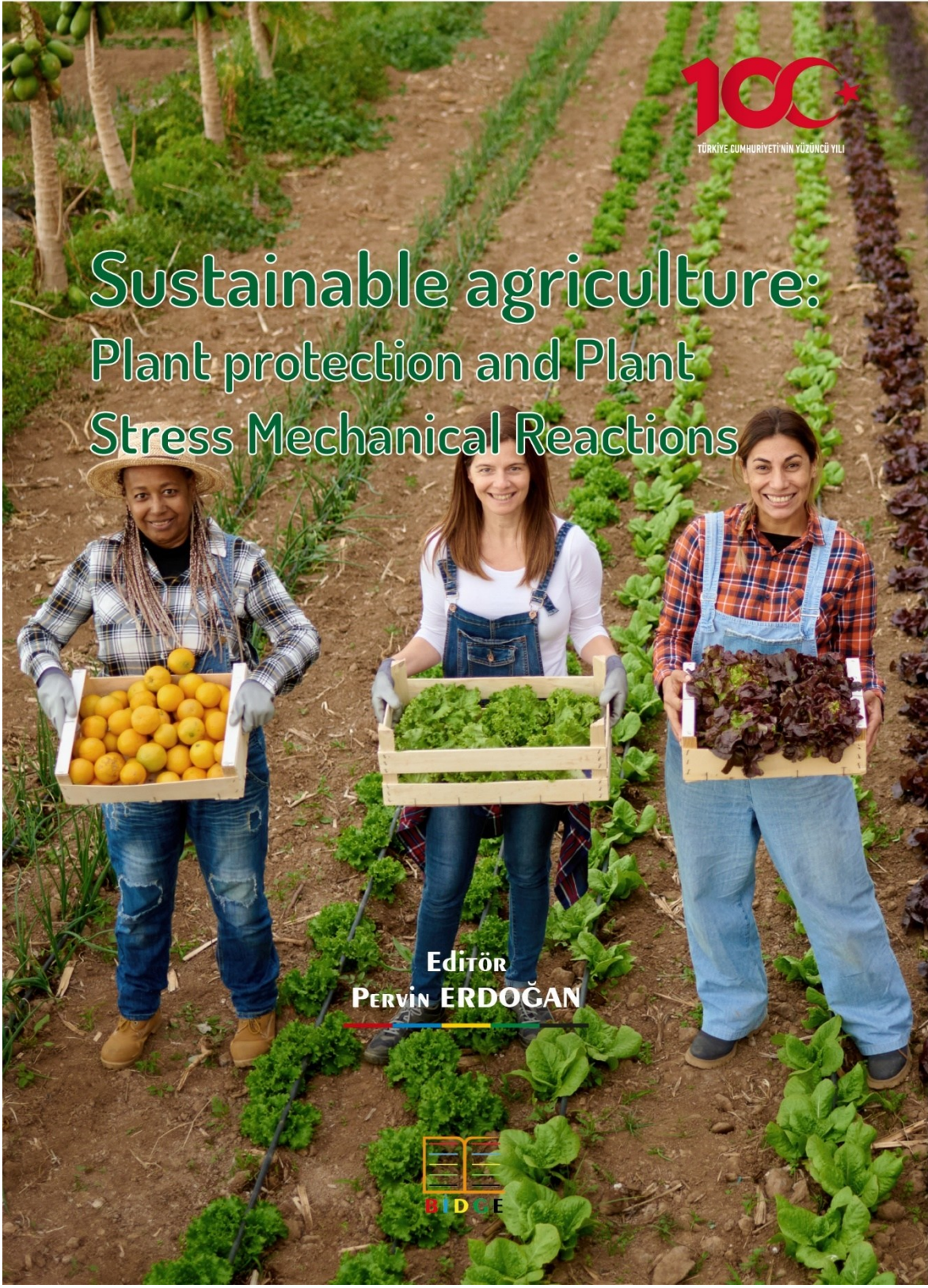

Sustainable agriculture: Plant protection and Plant Stress Mechanical Reactions

Editör
Pervin ERDOĞAN

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PREFACE

Rapid industrialization and population growth in the world in the second half of the twentieth century brought along significant environmental problems. As a solution, policies have been developed to eliminate the problem of hunger, and targets have been set to obtain high yields per unit area by using intensive inputs and to open new areas to agriculture.

In addition, intensive and unconscious use of pesticides and fertilizers, wrong tillage practices, residue risk, deterioration of the physical structure of the soil, loss of organic matter and vitality, disturbance of nutrient balance, salinization, and desertification have brought environmental problems such as. The use of fertile agricultural lands in flat areas for housing, industry, roads, and similar purposes has led to the opening of marginal areas with lower productivity to agriculture, which has triggered more intensive input use and further increased the problems. Moreover, agricultural areas are gradually decreasing due to abiotic and biotic factors, and yield and quality losses in the products obtained from these areas are also decreasing.

Today, intensive input agricultural practices in agricultural production areas to obtain more yield and quality products have created some undesirable effects, such as pollution of water and food resources, poisoning of non-target beneficial insects, and the development of insecticide-resistant insect populations. Therefore, the continuity of sustainable agricultural practices in the future will largely depend on the recognition of harmful factors in plant

protection studies within an ecological framework and finding and implementing the right solutions under their control.

Sustainable agriculture offers a potential solution to ensure that farming systems can feed a growing population in a changing environment. Given the limited supply of natural resources at any given cost and location, inefficient or harmful agriculture for the required resources can ultimately deplete available resources or the ability to afford and acquire them. Sustainable agriculture is not done in a single way. To date, different practices in different ways (integrated farming, organic farming, and good agricultural practices) have been subsumed under the umbrella of sustainability.

The aim is to keep the economy alive in the short and long term, to improve the quality of life of those engaged in agriculture, and to develop practices for this purpose while maintaining productivity in agriculture and reducing environmental damage. In fact, it can be considered a heading under which the solution proposals put forward to solve the problems created by industrial agriculture are gathered.

Sustainable agriculture means agriculture practiced in sustainable ways that meet the current food and textile needs of society without compromising the ability of future generations to meet their needs. Agriculture is the starting point of the food chain, which we define as primary production. Sustainable agriculture includes systems and practices that will improve the production of sufficient and high-quality food at affordable costs and the protection of farmland, farmers, the environment, and natural agricultural resources. It can be based on an ecosystem services approach. There are many ways to increase the sustainability of

agriculture. When developing agriculture within sustainable food systems, it is important to develop flexible business processes and farming practices. Agriculture has a very large environmental footprint; it causes environmental changes but is also affected by these changes.

Sustainable agriculture aims to protect human and environmental health and biodiversity, reduce chemical residues, restore the natural balance, and ensure the management of natural resources in a way that will enable continuous economic development while leaving a suitable and healthy environment for future generations.

As a result, healthy generations depend on healthy food, and healthy food depends on sustainable agriculture and plant protection activities.

Editor

Assoc. Prof. Dr. Pervin ERDOĞAN

Content

CHAPTER I

Examination of Plant Stress Mechanical Reactions at the Molecular and Cellular Level

Fadime KARABULUT[1]

1.Introduction

Stress hinders a plant's ability to function normally, which lowers the plant's development, yield, and quality of harvest. Environmental stresses upset the natural balance of defense mechanisms in plants (Mullineaux & Baker, 2010). Abiotic stresses that can harm plants include salt, drought, and heavy metals (HMs) (Faizan et al., 2023). Furthermore, the health of the soil significantly influences plant disease tolerance and resistance, despite genetic determination of these traits. Enough nutrient intake makes plants

[1] Dr., Firat University, Faculty of Science, Department of Biology, 23119, Elazıg/Türkiye, Orcid: 0000-0001-5186-2303, karabulutfadime9@gmail.com

more resilient to biotic stress and more tolerant of diseases (Shakeel et al., 2023).

Stressors in the environment cause cells to produce too many reactive oxygen species (ROS). Photosynthesis, photorespiration, and oxidative respiration are the three plant metabolisms that share cellular reservoirs of reducing and antioxidant enzymes when oxidative stress occurs (Noctor, De Paepe, & Foyer, 2007). All cell types engage in oxidative metabolism to some degree, and ROS production is an inevitable result of this process. Cellular reducing pools are typically replenished by the same processes, which counteract these effects.

An explosion of ROS can result from small environmental changes that quickly cause this delicate redox balance to shift (Li et al., 2009). The photosynthetic electron transport chain and the oxidation of water by light energy are the two processes that drive all photosynthetic organisms to function. Two closely spaced chlorophyll molecules make up Photosystem II (PSII), which functions as only one unit (Raszewski et al., 2008). When taken as a whole, they stand for the most oxidizing molecules in biology when it comes to the physical regulation that photosystems provide (Rappaport et al., 2002). Given that light is also the catalyst for chlorophyll oxidation, it is highly susceptible to daily fluctuations in light (Ruban, 2009). Proteins, lipids, and nucleic acids are examples of cellular macromolecules that must be protected from damage in order to preserve redox homeostasis. By properly buffering the change in PSII's overall redox capacity, this can be accomplished. Multiple, layered layers of reducing and antioxidant enzymatic systems are part of the evolved whole plant redox metabolism that balances PSII's strong oxidation potential. Reducing ROS damage

and regulating ROS signaling are two functions of endogenous plant antioxidant systems. In order to detoxify ROS or regenerate reductants, these systems usually contain both chemical and enzymatic reductants (Gill & Tuteja, 2010).

Because they do not regenerate well, reductants are usually kept in highly reduced states in extracellular spaces and vacuoles (Karpinska et al., 2018). Antioxidant systems react to stress, help plants sense their surroundings, and are connected to defense mechanisms in plants. Since antioxidants naturally resist stress and defend against pathogens, antioxidant systems could be useful in improving crop resilience and yield (Broad et al., 2020). Plants with higher medicinal value, better crop health, and innate pest resistance have been raised because of variations in antioxidant levels. One specific method for directly enhancing plant health and the external use of antioxidants is resistance (Smeriglio et al., 2016).

Oxidative stress occurs when a plant produces more ROS than it can scavenge. This causes surplus ROS to quickly leak to other plant sections. Plants are negatively impacted by high ROS levels, which can change a cell's morphology, metabolism, and physiological state. Peroxisomes, chloroplasts, and mitochondria are where plant cells generate the majority of ROS. Furthermore, ROS is a metabolic process byproduct. ROS possess distinct half-lives and oxidizing potentials (Miller et al., 2010). Through lipid peroxidation, these harmful compounds can oxidatively damage lipids, nucleic acids can affect DNA, and protein oxidation can inhibit enzymes, resulting in predetermined cell death (Apel & Hirt, 2004). Since plants cannot withstand high levels of ROS, to stop ROS-induced oxidative damage, a variety of enzymatic and non-enzymatic detoxification techniques have been developed. Antioxidants that are

not enzymatic include flavonoids, carotenoids, glutathione, and tocopherols. For the preservation of ROS homeostasis, both types of antioxidants are necessary (Mahmood et al., 2019). Research has indicated a connection between enhanced stress tolerance in plants and high levels of anti-oxidative activity (Faizan et al., 2023; Faizan et al., 2024).

Several types of plants have demonstrated that antioxidant applications can be used to alter the natural antioxidant systems of the plants and enhance their overall health. The mechanistic reactions of antioxidant compounds in plant species' ability to withstand stress are examined in this chapter.

2. Metabolism of reactive oxygen species (ROS)

When pathogen attack is detected by membrane receptor kinases, plasma membrane NADPH oxidases initiate the apoplastic ROS oxidative burst (Rahikainen et al., 2016). ROS release under control and callose accumulation have been identified as the main defense mechanisms in plant cells against any pathogenic invasion. Peroxidases that bind phenolic and glycoprotein components with hydrogen peroxidases have been found to be the source of the potentiation of these practices (Voigt, 2014). The internal hypersensitive response consists of fortifying the cell wall and then sending out signals to the defense signaling networks to initiate action and remove any fungi or other harmful elements from the intracellular and intercellular areas (Leon & Montesano, 2013). Plant resistance (PR) products cause elicitors to release primary and secondary signaling molecules, which in turn activate the entire signaling cascade (Gebrie, 2016). Numerous genes express themselves to create this intricate network sequence. Under stress

conditions, the well-known oxidative response is brought on by the WRKY53 transcriptional network (Triplett et al., 2016).

Through redox balances that increase transcription factor expression, the presence of ROS affects host-pathogen interactions, hormonal responses, and developmental processes (Barna et al., 2012). The production of ROS not only initiates ionic fluxes and phosphorylation of proteins, but it also cooperates with other signaling molecules like salicylic acid and nitric oxide (Torres et al., 2006). Salicylic acid and its transcription factors TGA and NRP1, as well as other well-known signaling network switches, are caused by ROS (Fu & Dong, 2013). NPR1 is typically found in the cytoplasm as a cysteine polymer once the enzyme S-nitroso-glutathione has nitrosylated it. However, during stress and when salicylic acid builds up, thioredoxins decrease disulfide bonds, and the redox state shifts (Lehmann et al., 2015). Even at longer accumulations, ROS can surprisingly occasionally only cause PR proteins rather than these salicylic acid-dependent signals, suggesting the existence of additional, as-yet-unidentified control mechanisms (Peleg-Grossman, Melamed-Book, & Levine, 2012). The superoxide ion is not as stable or diffusible as hydrogen peroxide for signaling. The mechanistic understanding of ROS and calcium ions is complex due to their co-production during signaling pathways and their tendency to be regulated by one another (Choi et al., 2017).

When photosynthetic activity is decreased and light excitation energy exceeds that needed for photosynthesis, ROS accumulation in chloroplasts is caused by abiotic stress situations (Toscano et al., 2019). Because ROS are produced by abiotic stressors that lower chlorophyll content and photosynthetic efficiency, they impede plant growth and reduce crop yield. The

degree of this harm depends on the type, frequency, and duration of the abiotic stress (Naing & Kim, 2021). In ideal circumstances, intracellular antioxidants neutralize ROS. When salt stress occurs, an excessive buildup of ROS results in oxidative stress, which profoundly disrupts normal metabolism and results in protein degradation and nucleic acid mutation (Czégény et al., 2014). Moreover, plants that produce too much ROS damage the integrity of their cell membranes by causing lipid peroxidation. Plant biochemical and physiological processes are consequently hampered. Malondialdehyde (MDA), the principal byproduct of membrane lipid peroxidation, is produced under stress in plants. The content displays the degree of cell membrane damage. The increase in this compound is used to gauge how well plants withstand salinity and is believed to be an indicator of oxidative stress (Yildirim et al., 2008; De Azevedo Neto et al., 2006). To reduce the oxidative damage caused by excessive ROS production, plants can use low-molecular-weight antioxidants such as tocopherol, carotenoids, reduced glutathione, ascorbate, and flavonoids (Pitzschke, Forzani, & Hirt, 2006). The three primary ROS-scavenger enzymes found in plants are POD, SOD, and CAT. CAT is used to break down hydrogen peroxide into oxygen and water. Similarly, SOD breaks down superoxide radicals to produce oxygen and H_2O_2. POD and a few additional enzymes convert H_2O_2 into harmless compounds (Radhakrishnan & Baek, 2017). Water is produced when H2O2 is reduced during the ascorbate degradation process by APX. The two enzymes that are best at scavenging active oxygen species that lead to oxidative stress are SOD and catalase. Peroxidases, which are present throughout the cell and have a much higher affinity for H_2O_2 than catalases, are another method of degrading H_2O_2 (Apel & Hirt,

2004). As an illustration, various studies have demonstrated that treating salt stress raises SOD activity (Kahrizi, Sedighi, & Sofalian, 2012). Under abiotic stress conditions, it was also discovered that the leaves of a number of plant species showed increased POD activity (Chookhampaeng, 2011). ROS are primarily produced by the electron transport chain (ETC) in mitochondria and chloroplasts in response to abiotic stress (Gill & Tuteja, 2010). This ROS production has many effects on plant metabolism (Figure 1).

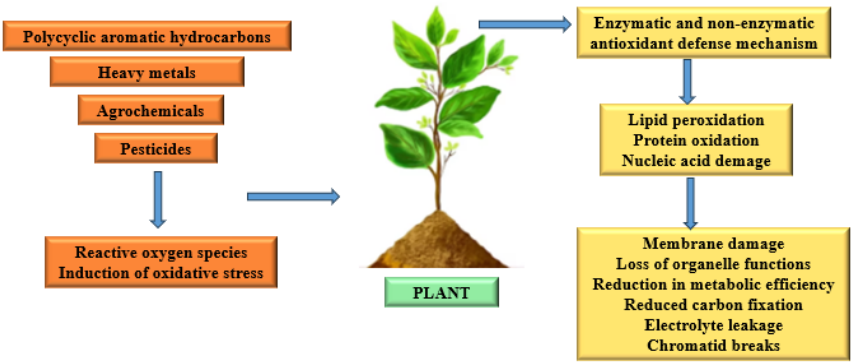

Figure 1. Manufacturing of oxidative stress and physiological impacts of abiotic stress in plants

3. Biomolecules under oxidative stress

When a cell's defense mechanisms are unable to keep up with the amount of reactive oxygen species (ROS), the cell is said to be in a state of oxidative stress, which can be eliminated. Under stressful conditions, plants can upset the equilibrium between the generation and removal of ROS. High levels of ROS can damage biomolecules, including DNA, proteins, and lipids. In the end, these reactions have the potential to destroy cells by altering the intrinsic properties of the membrane, such as fluidity, ion transport, DNA damage, loss of enzyme activity, and protein cross-linking (Apel & Hirt, 2004).

3.1 Changes in proteins

ROS-attacking proteins can cause them to change in a number of ways. The process of directly modifying a protein involves nitrosylating, carbonylating, disulfide bonding, and glutathionylating it. By conjugating with breakdown products of fatty acid peroxidation, proteins can undergo indirect modification (Yamauchi et al., 2008). A rise in ROS production can have a number of consequences, including the breaking apart of peptide chains, the alteration of particular amino acids, the buildup of cross-linked reaction products, and changes in electrical charge. Oxidative stress can harm tissues as a result of elevated levels of carbonylated proteins, a frequently used indicator of protein oxidation (Møller & Kristensen, 2004). Under a variety of stressors, plants have been shown to modify their proteins more (Maheshwari & Dubey, 2009). Peptides exhibit varied responses from different amino acids when attacked by ROS. Sulfur-containing and thiol-containing amino acid sites are the most susceptible to ROS attack. A thiyl radical that is created from remaining cysteine will form a disulfide bridge when active oxygen takes away a H atom from it through cross-linking with another thiyl radical (Stohs & Bagchi, 1995). Methionine sulfoxide derivatives can also be made by combining methionine and oxygen. Tyrosine can be cross-linked easily to create products containing bityrosine (Davies, 1987).

Iron-sulfur centers undergo irreversible oxidation when exposed to O2, which inactivates the enzyme (Gardner & Fridovich, 1991). Proteolytic digestion works better with proteins that have undergone oxidation. Protein oxidation has been proposed as a risk factor for ubiquitination, which makes a protein a target for proteasomal breakdown (Cabiscol et al., 2000). Raising H_2O_2 concentrations

during incubation led to an increase in the carbonyl content of Cd-treated plants, crude leaf extracts, and purified pea leaf peroxisomes. After being exposed to metals, oxidized proteins were broken down more quickly and had a 20% increase in proteolytic activity (Romero-Puertas et al., 2002). Numerous investigations have demonstrated that damage causes highly correlated and clumped products that, in addition to providing insufficient substrates for breakdown, can also prevent proteases from degrading more oxidized proteins after a certain point (Grune, Reinheckel, & Davies, 1997).

3.2 Oxidation of lipids

Lipid peroxidation increases in both cellular and organelle membranes when ROS levels surpass the threshold. Lipid-derived radicals produced by lipid peroxidation, which can react with proteins and DNA to cause damage, are a major source of oxidative stress. To determine whether ROS is causing damage to cell membranes under stressful conditions, lipid peroxidation levels are monitored. Therefore, in response to environmental stress, plants have been found to exhibit high levels of lipid peroxidation and degradation (Tanou, Molassiotis, & Diamantidis, 2009; Mishra, Jha, & Dubey, 2011). Rising ROS production and rising lipid peroxidation happen simultaneously. As phospholipids peroxidize unsaturated fatty acids, malondialdehyde (MDA) is produced as a byproduct that damages cell membranes (Halliwell & Gutteridge, 1989). In phospholipid molecules, ROS frequently target two regions. These two are the ester bond between glycerol and fatty acid and the unsaturated (double) bond between two carbon atoms. When it comes to ROS attacks on membrane phospholipids,

polyunsaturated fatty acids, or PUFAs, are particularly vulnerable. Peroxidation can occur in a large number of polyunsaturated fatty acids, starting with a single OH, since the actions involved in this process are components of a chain reaction that is cyclical. Phases one, two, and three comprise the entire lipid peroxidation process. The activation of O2, the rate limiter, is necessary for the first stage of lipid peroxidation. Polyunsaturated fatty acids' methylene groups react with O2 and OH to produce conjugated dienes, lipid peroxy radicals, and hydroperoxides (Smirnoff, 1995). ROS have the ability to break the chains of polyunsaturated fatty acids, thereby increasing the membrane's permeability and fluidity.

3.4 Effect on polynucleic acids

Animal physiology has studied oxidative damage to DNA in great detail since it plays a significant role in the emergence of cancer. Plant physiology has not given this issue enough attention because it does not pertain to plants that are significant for agriculture. Nonetheless, oxidative DNA damage can lead to crop plant mortality and the senescence of seed stocks (Britt, 1996). Conventionally, lesions are classified into three categories: chemically modified bases, mismatched bases, and double-strand breaks (Yoshiyama, Sakaguchi, & Kimura, 2013). Polynucleic acid degradation is primarily caused by hydroxyl radicals, which attach themselves to nucleotide base double bonds and remove hydrogen ions from each methyl group in thymine and the C-H bonds in 2-deoxyribose. Plants have repair mechanisms that include base and nucleotide exchange in addition to direct molecular repair of the damaged area (Yoshiyama, Sakaguchi, & Kimura, 2013). Increasing the cytosolic and organelle antioxidant defenses is another aspect of

protection. Peroxiredoxin and glutathione, two nuclear ROS scavengers, have been shown to be insufficient to protect DNA from oxidative stress (Vanderauwera et al., 2011). They demonstrated the critical roles that cytosolic ascorbate peroxidase and catalase play in shielding nuclear DNA from damage. Moreover, signaling pathways and particular transcription factors are activated by ROS-induced DNA damage, resulting in DNA repair (Yoshiyama, Sakaguchi, & Kimura, 2013).

3.5 Impect on carbohydrates

The most prevalent class of organic molecules in plants, carbohydrates have also received the least attention in terms of research on their function in stress signaling and oxidative damage. In addition to supplying non-enzymatic antioxidant defense (mannitol, cellulose, and sucrose), they also play crucial roles in the mechanical formation and support of plant cells (cellulose, pectin, and so on), the regulation of enzyme activity and osmotic pressure (low molecular weight sugars), and the storage of reduced carbon. Carbohydrate oxidation may be detrimental to plants. Cu^{2+}-produced OH breaks down and loosens the cell walls of xyloglucans and pectins through a non-enzymatic reaction (Fry, Miller, & Dumville, 2002). This may help cells proliferate and expand while also accelerating the ripening of fruit (Fry, Miller, & Dumville, 2002). In stressful conditions, when catalytic Cu and Fe activities multiply several times, this reaction, while beneficial, may have pathophysiological effects (Moran et al., 1994). ROS scavengers are most likely mono- and disaccharides (Couée et al., 2006). According to EPR and HPLC testing, OH scavenging abilities are as follows: Maltose> sucrose> fructose> glucose> deoxyribose> sorbitol

(Morelli et al., 2003). Except for formate, the metabolism of the other products synthesized in these reactions is unknown. Specific carbohydrates, like mannitol, have been associated with an increase in the resistance of several species to oxidative stress (Couée et al., 2006). Rather than being major targets for ROS or redox switches, they most likely function as structural, osmotic, nutritional, and signaling agents (Couée et al., 2006).

3.5 Effects on phytohormones

Plant development, growth, and stress reactions are regulated by phytohormones (Verma, Ravindran & Kumar, 2016). Plant stem remodelling is a particularly effective use of phytohormonal responses due to the crosstalk between their wide-ranging effects. Furthermore, the synthesis of ethylene, ABA, and GAs depends on the antioxidant ascorbate, which means that the presence of natural antioxidants is inherently connected to the synthesis of phytohormones (Gallie, 2013). Antioxidant application from outside sources thus frequently modifies the levels of phytohormones. There are two phytohormones involved: those related to stress, SA, ABA, ethylene, and JA (Bari & Jones 2009), and those that promote growth, auxin, GA, and cytokinin (Verma, Ravindran & Kumar, 2016). The "growth" or "stress" events are probably significantly influenced by these phytohormones. Plant growth is increased by most antioxidant applications. It has been demonstrated that applying glutathione, ascorbate, and GABA separately raises the levels of auxin in Arabidopsis, cucumber, citrus (*Citrus sinensis*), and maize (Guo et al., 2020). In Arabidopsis, treatments with ascorbate and melatonin lower their levels (Ren, Rutto & Katuuramu, 2019). Both growth and development are influenced by plant hormones, so the plant's growth stage most likely determines

some or all of their effects. Phytohormone changes in maize appear to be growth stage dependent when auxin levels are assessed and ascorbate is administered (Dinler, Demir & Kompe, 2014).

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same treatments also typically promote growth in stressful situations. Exogenous antioxidant therapies also frequently influence the levels of phytohormones linked to stress in a comparable way (Ji et al., 2018; Xu et al., 2018). In fact, there has been a suggestion that, in the case of allantoin application, increased stress tolerance is achieved through the primary molecular mechanism of ABA activation (Watanabe et al., 2014). Pre-applying ABA makes certain species more resistant to abiotic stress. It is well known that abiotic stressors cause plants to produce higher levels of ABA (Olds, Glennon, & Luckhart, 2018). Plants under biotic or environmental stress exhibited elevated SA levels (Hayat et al., 2010). It is thought that through SA-based modifications, BTH affects plants' ability to withstand biotic stress. BTH is regarded as a functional analogue of SA (Klessig, Choi, & Dempsey, 2018). Additionally, in Arabidopsis, maize, and citrus, SA levels rose in response to treatments with GABA, glutathione, and ascorbate (Sultana & Chattopadhyay, 2020). It has been observed that when ascorbate levels are genetically reduced, SA levels increase, and when glutathione levels are genetically reduced, SA levels decrease (Brosche & Kangasjarvi, 2012). Because of the H2O2 levels, SA might be a sensitive gauge of a cell's antioxidant state (Brosche & Kangasjarvi, 2012). Compared to SA and ABA, ethylene and JA amounts are measured more rarely. Nevertheless, in citrus and Arabidopsis, glutathione, GABA, or allantoin treatment was found to raise JA levels (Hijaz, Nehela, & Killiny, 2018). The elevated concentrations of these phytohormones that are sensitive to stress vary somewhat. Again, ABA and SA levels are influenced by growth stage, plant species, and sample collection time relative to application time (Dinler, Demir, & Kompe, 2014). In conclusion, the

examination of phytohormone levels confirms the finding that antioxidants applied externally are more beneficial when under stress.

4. Impacts and consequences of stress on plants and defense against oxidative stress

The defense mechanisms of plants against oxidative stress, namely ROS scavenging and cell repair systems, have been the subject of in-depth studies and multiple reviews in recent times (Farmer & Mueller, 2013). Numerous defense systems protect plants from oxidative stress (Gill & Tuteja, 2010). Firstly, ROS and free radicals are scavenged (removed) by enzymes and non-enzymatic substances that are synthesized from scratch and then anactivated. The subsequent items are important enzymatic antioxidants that exhibit strong affinity for specific ROS: cytosolic Cu-Zn-SOD, mitochondrial Mn-SOD, chloroplastal Fe-SOD (all SODs: superoxide + 2H+⟶ H2O2+ O2), catalases (2H2O2⟶ 2H2O + O2), peroxidases (R/HOOH + R-H2⟶ R + 2H2O/ROH), peroxiredoxins (ROOH → ROH), thioredoxins, and glutaredoxins (both: R-S-S-R → 2R-SH). Non-enzymatic antioxidants that are not specific to any particular ROS. Furthermore, ROS are shielded by a class of enzymes. Peptins and other cell wall polysaccharides, phytochelatines (glutathione oligomers), metollothioneines (small Cys-rich proteins), and structural proteins are among the substances that reduce the catalytic activity of transition metals and are therefore the first line of defense against them (Zagorchev et al., 2013). Second, less oxidatively susceptible lipids and protein isoforms are most likely synthesized by plants. In order to activate particular genetic or metabolic pathways, this requires an upstream signaling step (Myouga et al. 2008). Third, the majority of plants produce fast-

dead cell layers that are most likely caused by ROS-induced mechanisms designed to shield live tissues from stress (Demidchik et al., 2010). They offer protection from pathogens and hostile substances. In order to replace broken parts, plants also activate their biosynthesis systems (Yoshiyama, Sakaguchi, & Kimura, 2013).

The species of plant, its developmental stage, and the salt concentration are some of the variables that affect how quickly plants shrink (Yadav et al., 2019). Salt stress inhibits plant growth, just like many other abiotic stresses do. Plants can withstand salt stress by using stunted growth as an adaptive survival mechanism (Munns, 2002). Because salt stress can lower the expression of important regulatory genes (like cyclin and cyclin-dependent kinase) involved in the progression of the cell cycle, it can affect a plant's capacity to efficiently absorb nutrients and water. This can lead to a reduction in the number of cells in the meristem and growth inhibition (Chinnusamy & Zhu, 2003). Plants convert solar energy into chemical energy through a process called photosynthesis, which is fundamental to their biology. Salt-induced declines in photosynthetic activity are linked to a number of factors, including reduced CO_2 supply, stomatal closure, altered enzymatic activity, and damaged photosynthetic apparatus. As the amount of chlorophyll decreases, more chlorophyll is oxidized and degraded, which is caused by an increase in ROS (Qin et al., 2020; Al Hinai et al., 2022). Excessive ROS production is caused by pseudocyclic electron transport, which is the result of the electron transport chain being inhibited. ROS affect photosynthetic proteins and photosystem assembly as a result (Zahra et al., 2021). When plants become dehydrated, they produce large amounts of the quaternary ammonium compound glycine betaine (Zhu et al., 2022). Glycine

betaine is made in the chloroplast. Here, it builds up and aids in maintaining photosynthetic efficiency by osmotically regulating the thylakoid membrane. Glycine betaine has been thoroughly investigated using transgenic methods to alter its metabolic pathways (Annunziata et al., 2019).

In reaction to abiotic stress, proline is increased because it is an essential compatible osmolyte and antioxidant. A physiological hallmark of a plant's response to abiotic stress has been identified as high proline content (Abdelhamid et al., 2013). Salinity stress causes proline to be produced and accumulated in plant cells by activating the genes involved in proline biosynthesis (Kim & Nam, 2013). Catalyzing the first step of proline biosynthesis is the enzyme Pyrroline-5 carboxylate synthetase (P5CS). One of P5CS's two isoforms is P5CS1.

The electron transport chains of mitochondria and chloroplasts are most likely the source of abiotically induced ROS, which are detoxified by antioxidant and non-enzymatic enzymes found in plants. Non-enzymatic antioxidant molecules include glutathione (GSH), ascorbates (ASC), and carotenoids. Conversely, ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) are examples of antioxidant enzymes (Azeem et al., 2022) (Figure 2).

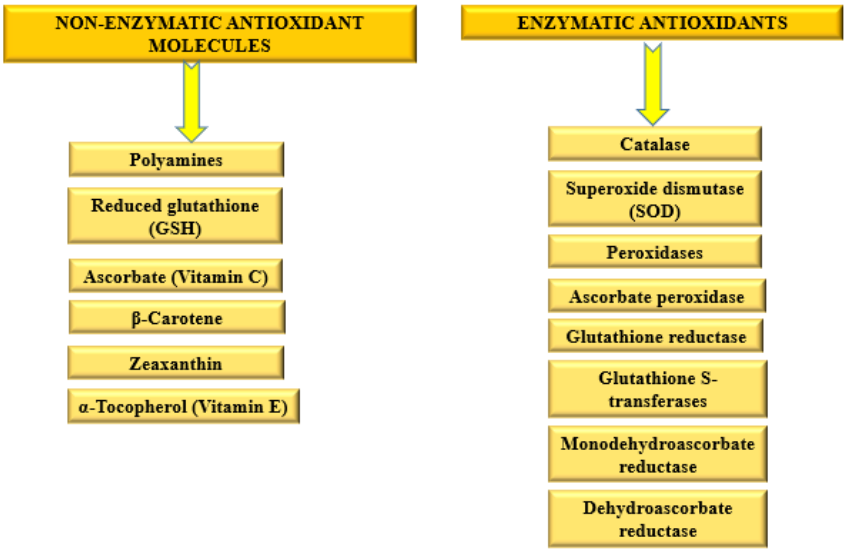

Figure 2. Antioxidants containing both enzymes and non-enzymes

High concentrations of ROS are neutralized or scavenged under abiotic stress conditions by these antioxidant molecules and enzymes. First, SOD is produced by plant cells, which starts the sequential detoxification process. The first line of defense for the body against superoxide radicals is SOD, which converts them into oxygen and hydrogen peroxide. As such, it reduces hydroxyl radicals. Metal ions (Fe^{3+} and Cu^{2+}) are lowered by superoxide radicals, which cause the production of hydroxyl radicals, which have the potency to oxidize lipids and cellular membranes. Following production under abiotic stress, POX and CAT break down hydrogen peroxides and their derivatives (Qamer et al., 2021). The conclusion reached was that reduced membrane lipid peroxidation is linked to decreased MDA production in abiotic stress-tolerant plants (Hussain et al., 2022). In conclusion, it demonstrates the protective role of several chemicals, pigments, and

enzymes, which lower oxidative damage and raise plant tolerance to stress.

Plant hormones, or phytohormones, are a crucial class of endogenous regulatory molecules that are responsible for regulating the growth and development of plants. Plants' phytohormone-mediated stress tolerance is mediated by nine distinct and well-characterized groups of hormones. The hormones have complex functions in plants. These hormones are classified as growth-promoting hormones (SLs), brassinosteroids (BRs), auxin, GA, cytokinins, SA, and JA as stress-responsive hormones (Verma, Ravindran, & Kumar, 2016). Because of their complex interactions, phytohormones allow stress response mechanisms to be attributed to more than one hormone (Ku et al., 2018). One essential hormone that acts as a central integrator in response to stress is ABA, which triggers an adaptive signaling cascade and modifies gene expression. A kinase cascade is triggered by the instantaneous elevation of endogenous ABA levels following stress exposure (Chen et al., 2020).

5. Using transgenic methods to increase oxidative

A vital component of guaranteeing global food security is sustainable agricultural production. Crop growth and yield, however, are impacted by a variety of stress conditions. Creating stress-tolerant plants is a crucial first step towards overcoming these stressful environments. Developing stress-tolerant plants may benefit from an understanding of how each gene functions under various stress situations. Transgenic plants with increased resistance to various environmental stressors are created by overexpressing different genes that encode different antioxidant enzymes. Many

plants that are resistant to stress have been created through genetic modification in recent times.
. Most of this research has focused on abiotic stress; however, there aren't many reports that explain how these enzymes function when facing biotic stress. Furthermore, a strong promoter in transgenic lines leads to an overexpression of the antioxidant enzyme gene in all of these investigations. As a result, the plant has a higher potential for tolerance to stressful situations. Therefore, these results are critical to the development of stress-tolerant plants, and the knowledge gained is useful for the productive and sustainable growth of a variety of crops under a range of environmental circumstances. The majority of transgenic techniques currently used in various plants lack a comprehensive strategy. Only one gene's expression under a potent promoter and a particular kind of stress have been the subject of these investigations. To learn more about how plants react to different stress situations, more studies looking into the gene pyramid approach are necessary. Moreover, the majority of research on transgenic overexpression is associated with the model plant Arabidopsis. Consequently, in order to ensure the security of food worldwide, genetic engineering must move towards cash crops. Comparably, only a small number of transgenic studies have offered details on how energy is reallocated and how changes in gene expression profiles for improved stress tolerance can either increase or decrease crop yield (Poli et al., 2018).

It is now better known how ROS-scavenging systems contribute to plant stress tolerance thanks to the application of gene transfer technology to change a plant's stress tolerance. Increasing plants' ROS-scavenging capabilities through transgenic methods has been clearly demonstrated in numerous studies to provide some

protection against oxidative damage, indicating that plants that employ this approach are more resilient to stress (Ruan, Shao, & Teixeira da Silva, 2012). Genetic engineering of specific genes has been explored as a means of achieving tolerance in a variety of efficient ways that result in increased productivity, better antioxidant defense, and increased tolerance to abiotic stress. The levels of both enzymatic and non-enzymatic antioxidant defense system components are generally significantly higher in transgenic plants as compared to non-transformed or WT plants. In contrast to non-transgenic tubers, transgenic potatoes (*Solanum tuberosum* L. cv. Taedong Valley) with the l-gulono-c-lactone oxidase (GLOase) gene overexpressed exhibited greater basal AsA content (141%) and were more resilient to methyl viologen-induced abiotic stressors. Furthermore, a direct relationship was observed between the transgenics' high AsA accumulation levels and their resistance to abiotic stressors. Plants with changed GSH levels were also found to be more stress-tolerant (Reisinger et al., 2008). It's been suggested that overexpressing α-tocopherol may make plants more resistant to abiotic stresses that cause oxidative stress.

Tocopherol cyclase (VTE1), the enzyme that catalyzes the penultimate stage of tocopherol synthesis, is encoded by the VTE1 gene. Transgenic tobacco plants expressing overexpressed VTE1 from Arabidopsis demonstrated decreased levels of H_2O_2 content, lipid peroxidation, and electrolyte leakage following a 20% PEG drought (Liu et al., 2008). More resistance to high temperatures (40 and 45 °C) was demonstrated by SOD-overexpressed (SOD-OX) leaves than by non-SOD-OX lines after 30 minutes and 2 to 24 hours of exposure (Artlip et al., 2009). Sweet potato (Ipomoea batatas) plants were better able to recover from drought stress when

Cu/ZnSOD and APX were expressed in their chloroplasts (Lu, Deng, & Kwak, 2010). Transgenic plants significantly upregulated the expression of antioxidant enzymes (SOD, APX, and CAT) in response to drought stress when compared to non-transgenic plants, and periodic rewatering decreased MDA levels and electrolyte leakiness. Compared to non-transgenic plants, transgenic plants under drought stress showed improved growth, photosynthetic activity (Fv/Fm), and water status. Because GST and CAT1 genes are co-expressed in transgenic rice seedlings exposed to both salt (200 mM NaCl) and paraquat, the transgenic plants only significantly increased their GST activity in response to paraquat stress (Zhao & Zhang, 2006). When given identical conditions, transgenics produced less H_2O_2 and MDA than non-transgenics. They came to the conclusion that elevated CAT and GST activity as well as a combined increase in SOD activity might be responsible for the improvement of the ROS scavenging system that results in enhanced oxidative stress protection in GST and CAT1-transgenic rice plants. Transgenic Brassica juncea plants with enhanced tolerance to Cd stress have been linked to overexpression of the BjCAT3 gene, whose CAT activity is roughly two times higher than that of WT plants (Guan et al., 2009). In order to create transgenic plants with increased resistance to a variety of environmental stresses, it was discovered that simultaneous expression of multiple antioxidant enzymes, such as CuZnSOD, APX, and DHAR, in chloroplasts was more effective than single or double expression (Lee et al., 2007).

Abiotic stress (mechanical damage) was more tolerable for transgenic tomatoes with increased GPX activity, but biotic stress was less robust (Herbette et al., 2011). Additionally, they reported

that the overexpression of GPX results in different outcomes when faced with biotic and abiotic challenges, indicating that the regulation of both forms of stress responses depends on this scavenger enzyme. It is more difficult to regulate and engineer plant resistance to abiotic stresses because it is genetically complex and multigenic. In order to adapt to abiotic stress, plants express genes that are involved in regulatory and signaling pathways, genes that encode proteins that are resistant to stress, or enzymes that are involved in the processes that result in the synthesis of chemical and functional metabolites (Vinocur & Altman, 2005). A slight improvement in stress tolerance has been achieved, despite the fact that overexpressing a single antioxidant defense system component can result in partial tolerance to a particular abiotic stress (Lee, Kwon, & Kim, 2009). Overexpressing a single enzyme is insufficient to stabilize ROS levels due to the coordinated nature of the ROS detoxification system. In the field of biotechnology, transgenic plants are very helpful instruments. It will further enhance our comprehension of the molecular physiology and gene networks that underlie plant responses to abiotic stressors.

6. Conclusions and future prospective

Finding effective methods to boost plants' stress tolerance. It is critical to make progress in comprehending the physiological and molecular mechanisms underlying environmental stress tolerance. Plants require ROS production, metabolism, and detoxification in order to complete their life cycle. Also, as a signaling molecule, ROS are involved in a number of plant functions. Understanding the reasons behind and mechanisms by which environmental stressors negatively impact plants requires research on plant defense and tolerance mechanisms. Thanks to developments in genetic

techniques, significant progress has been made in producing transgenic lines with altered antioxidant levels or conventional lines with enhanced oxidative stress tolerance. By enhancing their anti-oxidative system, protectants applied topically to plants can also fortify their defenses and reduce oxidative stress. Antioxidant enzyme overexpression is one way to increase stress tolerance in transgenic plants. Several mechanisms exist for applying antioxidants to different plants to enhance their growth, development, and resistance to stress. Various antioxidant applications are associated with several common direct effects on naturally occurring antioxidant chemical and enzyme systems: enhanced photosynthetic resilience or capacity, altered antioxidant metabolism, and modified phytohormone signaling. In conclusion, research on it can be done by contrasting applications of reducing antioxidants with antioxidants that primarily influence endogenous antioxidant systems (BTH, proline, and GABA). Comprehending this concordance will require more research. Before the growth advantages of antioxidant application in agriculture can be realized in large-scale use, crucial information regarding the mechanisms by which antioxidants produce their effects and the safety of their use is also required.

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CHAPTER II

New Approaches to Control Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) (Coleoptera : Chrysomelidae)

Pervin ERDOĞAN[1]

Introduction

The potato *Solanum tuberosum* (L.) plant originates from the high plateaus of the Andes mountains in South America. Cultivated by the natives more than 2000 years before the Spanish invasion of this continent, the potato was first brought to Europe by Spanish sailors. The potato, which is known to have been sold in Seville in 1573, was later recognized and cultivated in other European countries. Especially in countries like Ireland, whose climate is suitable for potato cultivation, it has gradually become a staple food for people. In fact, when the Potato Late Blight (*Phytophthora*

[1] Doç. Dr. Pervin ERDOĞAN, Sivas Bilim ve Teknoloji Üniversitesi, Tarım Bilimleri ve Teknoloji Fakültesi, Bitki Koruma Bölümü, Sivas/Türkiye, Orcid: 0000-0001-5553-4876, pervinerdogan@gmail.com

infestans (Mont.de Bary) (Peronosporaceae), which started in 1840 and lasted for several years, completely destroyed the crop, one million people died from starvation and disease, and 1.5 million emigrated to America. The potato was taken to the other continents of the world from Europe during the colonial movement (Kuşman et al., 1988).

Potatoes rank fourth after rice, wheat, and maize among the main food sources for the world's population. It is a product of high nutritional value, rich in carbohydrates, protein, vitamins, and minerals. It is a valuable food source, especially in underdeveloped countries where malnutrition is widespread. In addition to human and animal nutrition, potatoes are also used as raw materials for industry. Potato is a product that has advantages such as its ability to adapt quickly to different climatic zones, its ability to be grown in almost every region of the world, its higher yield per unit area, and its high nutritional value (Kolsarıcı, 2011). The widespread use of the potato, which is a staple food product, rapidly increases its consumption. Potato production in the world was realized at 359 million tons on 16.5 million ha in 2020 (Anonymous, 2022).

There are many pests that cause crop loss in potato cultivation, such as the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Col.: Chrysomelidae), the potato tuber moth (*Phthorimae operculella* Zeller, Lep.: Gelehiidae), *Agriotes* spp., *Agrotis spp., and* Aphidis. The most common and important of these is the Colorado potato beetle

Leptinotarsa decemlineata

Members of the genus *Leptinotarsa*, which includes at least ten species found north of Mexico, true potato beetles are widespread in North and South America and number over 40. Two species are found in the eastern states or across most of the United States, although most species found north of Mexico are found in the southwestern region of the country. Of these two, CPB is the most well-known because it poses a major threat to potatoes and other solanaceous plants (Arnett, 2002).

With the exception of Florida, Alaska, California, Hawaii, and Nevada, the majority of the United States and Mexico are home to the CPB Although it was initially discovered in Florida in 1920, it is not usually a significant problem. It is a nuisance throughout Central America and can even be found in southern Canada. Europe and several regions of Asia have received introductions of the species (Capinera, 2001).

Description

The adult is yellowish orange with a curved back. Adult beetles are typically 6–11 mm in length and 3 mm in width. The beetles have ten distinct black stripes on their elytra, giving them an orange-yellow color. The eggs are yellow to orange, and are about 1 mm long. The orange-pink larvae can grow to a maximum length of 15 mm during their final instar stage. They feature a big, 9-segmented abdomen, a black head, and conspicuous spiracles. There are four instar stages in the beetle larva. The pronotum of the larvae changes color from black in the first and second instars to an orange-brown edge in the third instar. The head stays black during these

stages. The pronotum of larvae in their fourth instar is roughly half light brown in color.

Pupae, after digging two to five centimeters into the ground, start to pupate after two days. The round, orange-colored pupae of the Colorado potato beetle are. 5.8 is the average development time (Wilkerson et al., 2005; Capinera, 2001).

Life Cycle

The female CPB can lay over 500 eggs in four to five weeks, making them extremely productive (Capinera, 2001; Rasin, 2017). The eggs are approximately long and range in color from yellow to orange. Usually, they are placed on the underside of host leaves in groups of roughly thirty. Temperature affects how all life stages develop. The eggs hatch into reddish-brown larvae with humped backs and two rows of dark brown markings, one on each side, after 4 to 15 days. Their host plants' leaves serve as their food source. Larvae develop into four different growth phases, or instars. The length of the first instars is around 1.50 mm, while the fourth and final instars are approximately 8 mm. The first through third instars each last about 2–3 days; the fourth lasts 4–7 days. Upon reaching full size, each fourth instar spends several days as a nonfeeding prepupa, which can be recognized by its inactivity and lighter coloration. The prepupae drop to the soil and burrow to a depth of several inches, then pupate. In 5 to 10 days, the adult beetle emerges to feed and mate. This beetle can thus go from egg to adult in as little as 21 days. The adults may go into diapause and wait until spring to emerge, depending on the temperature, light, and quality of the host. After that, they go back to their host plants to mate and feed; adults that overwinter might start mating as soon as the spring emerges.

Every growing season, there may be three or more generations in some places (Ferro et al., 1999, Capinera, 2001; Wilkerson et al., 2005; Rasin, 2017).

Hosts

The CPB prefers to feed on potatoes, but it can also live on a variety of other plants in the Solanaceae family, such as belladonna, eggplant, ground cherry, henbane, horse nettle, pepper (occasionally), tobacco, thorn apple, tomato, and buffalo-bur, which was the plant it was first known to feed on. The ability of the CPB of the CPB to adapt its host range to locally abundant *Solanum* species has been demonstrated. Non-solanaceous plants are not usually regarded as regular hosts for CPB, although they do occasionally feed on them (Capinera, 2001).

Alternative Control Methods to Chemical Insecticides

Chemical therapies, cultural customs, and biological control are some of the current methods used to manage and control CPB (Maharijaya & Vosman, 2015). The majority of CPB management methods, both past and present, have depended on pesticides (Grafius & Douches, 2008). Despite the fact that the use of pesticides led to a sharp decline in CPB populations, resistance to the active ingredients developed. The fact that CPB has grown resistant to the majority of approved pesticides is now widely known (Scott et al., 2005). Other control measures are needed because CPB has become resistant to every major class of chemical insecticide.

Cultural practise

Many cultural practices have been implemented to reduce the potato beetle population, such as rotation, manipulation of planting

time and crop varieties, use of mulch, cover crops, and trap crops (Hough-Goldstain et al., 1993). Alternation in the control of potato beetles was first proposed in 1872 (Bethune, 1872), and this method not only achieved good results in the control of the pest but also ensured the control of many diseases and weeds (Casagrande, 1987). The density of egg packets in the rotation area was 10% lower than in the non-rotation area (Lashomb & Ng, 1984). Rotation of the potato plant with a non-host plant (wheat) reduced Colorado potato beetle adult density by 95.8% in the early season. Late and early planting was aimed at suppressing the second instar larval population. Summer adults appear in the late season in late-planted crops. The short-day period stimulates reproductive diapause and largely eliminates the effect of second-generation larvae on the crop. In addition, early planting eliminates the larvae of the second generation. In this case, the crop that is ready for harvest is harvested.

Another cultural method of CPB is the application of mulch. The L. *decemlineata* larval population was significantly reduced in potato (Stoner, 1993) and eggplant in the parcels mulched with straw. It was observed that the larval population (1st and 2nd periods) peaked 1-2 weeks later in the mulched field compared to the non-mulched field. In addition, mulch increases the time *L. decemlineata* needs to find the plant (Ng & Lashomb, 1983). Increases the probability of adults leaving the area (Weber et al., 1994). In general, a six- to ten-cm layer of wheat straw reduced defoliation in potatoes by 2–2.5 times (Brust, 1994). One good way to lower your risk of Colorado potato beetles is to rotate your crops. A potato field's chance of damage is significantly reduced if it is situated further away from a potato crop from the previous year. This

is because beetles will colonize the field later in the season. Colorado potato bugs require 21.11 °C to take flight (Anonymous, 2023). In the fall, the potato harvest should be complete, and no tubers should be left in the field. Otherwise, the remaining tubers grow green the following year and become a source of nutrition for the adults emerging from the overwintering site. In the spring, the areas planted the previous year should be checked, and the remaining potato plants should be destroyed along with the insects on them (Anonymous, 2023).

Mechanical control

Because of their size, the potato beetle's adults and larvae are easily visible. In small areas, collecting and destroying adults and crushing their eggs is highly effective.

Because wood ash is poisonous to both adults and larval stages, laboratory assays have identified it as a potential chemical for managing CPB. All stages of beetles were destroyed when they were exposed to wood ash on a permanent basis for up to ten days. The primary issues that were identified were the declining effectiveness with repeated use and the declining field activity in damp settings. However, the study's author proposed that thick ash layers placed in strips around the bases of potato plants could function as a physical barrier similar to a fence, preventing large beetle colonization since CPBs would avoid crossing it (Boiteau et al., 2012). Physical control is an additional option to reduce CPB numbers. The development of plants and insects can be controlled at different stages by using pneumatic and thermal pest controls (Laguë et al., 1999). Here, scientists take advantage of the theory that early-

season potato plants, which are still growing out of the ground, are less susceptible to heat than beetles and their eggs.

Burners that burn propane are aimed at the rows of crops, killing most bugs while leaving the plants relatively healthy. On warm, sunny days, when CPBs are more active and frequently feed on the tops of potato plants, the maximum control efficacy was seen. Flaming can be a fairly effective pesticide when compared to the most common ones, which typically control 25–50% of overwintering adults. In field experiments, burning beetles resulted in up to a 90% death rate and a 30% decrease in the number of eggs that hatched (Moyer et al., 1992).

Biological control

Predators and Parasitoids

Many predators and parasites feed on CPB (Hough-Goldstain et al., 1993). The pink-spotted lady beetle, *Coleomegilla maculata* De Geer, which feeds on the eggs and larvae of cpb, consumes 37.8% of the eggs laid by first-generation adults and 58.1% of the second generation (Groden et al., 1990; Hazzard et al., 1991) (Figure 1. a). It feeds on the larvae of *Perilus bioculatus* and *Padisus meculiventris* CPB (Figures, b, c). These released predators suppress 62% of *L. decemlineata* (Biever & Chauvin, 1992), reducing leaf consumption to 86% (Hough-Goldstain et al., 1993; Goldstain and McPherson, 1996), and increasing potato yield by 65% compared to the control (Biever & Chauvin, 1992). *Lebia grandis* adults parasitize CPB pupae in the soil while feeding on their eggs and larvae (Figure 1. d, e, f) (Weber et al., 2006). It was determined that *Edovum puttleri* CPB killed 67–79% of the egg packet and parasitized 71–91% of the eggs (Lashomb et al., 1987).

It was revealed that the parasitism rate in potato plants was generally low and rarely exceeded 50% (Van Drieshe et al., 1991). It is emphasized that the performance of *E. puttleri* in the field could be much further improved by using an artificially defined carbohydrate source (Idone & Ferro, 1990). In some countries, *E. puttleri* is also commercially available.

Figure 1. *a) Cleomegilla maculata, b) Perilus bioculatus, c) Pedisus maculiventri, d) Labia grandis, e) L. grandis prepupa with the host, f) L. grandis first instar with the hoost. (https://www.google.com/search?+predator&tbm)*

Studies have shown that 50% of the eggs of CPB in potato plants are parasitized by *E. puttleri,* while this rate increases up to 91% in potatoes. It was noted that if potatoes and eggplants are grown where CPB is a problem, *E. puttleri* can be released for control. It was revealed that the first release should be done when the first egg packets are seen, and the second release should be done when the second egg packet is seen in early summer. The period of the second release coincides with the period of heavy damage. There are rare predators that feed on CPB. It is known that 14 species of the family Carabidae, 3 species of the family Coccinellidae, and the mite *Xyaticus kochi* have been feeding on CPB in the Soviet Union (Sorokin, 1976). Eight species of the genus *Labia* and five species of other insects are recorded feeding on CPB in Mexico (Logan, 1990). *Pterorostichus chalcites* was observed to feed on CPB (Hampel & Hough-Golstain, 1992). *Phalangium opiliopreys* were reported to feed on the eggs and early larvae of CPB (Drummound et al., 1990). In addition, Agasyeva (2023) suggested that *P. bioculatus* had an entomophage an entomophage efficiency of about 98% on CPB. CPB was also consumed by other insects besides *P. bioculatus*. *Zicrona caerulea* L., *Polistes gallicus* L., members of the Coccinellidae family, and others are noteworthy among them. The most significant parasitoid is *Myiopharus doryphorae* (Riley), a tachinid fly that affects the final generation of beetles and grows to great concentrations in the fall. Early-season parasitism rates in Colorado keep *Leptinotarsa decemlineata* from developing into a significant pest (Capinera, 2001).

Microbial Insecticides

Natural enemies can suppress the potato beetle population, which has a high reproductive power. Today, in the absence of pesticides, natural enemies keep potato beetle density below the economic damage threshold (Ferro, 1985). *Beauveria bassiana* (Hyphomycetes), a pathogenic fungus, affects many insect species, including the potato beetle. (Figure 2. a, b, c) Yıldırım et al. (2023) demonstrated that *B. bassiana* can be used against CPB. In the same study, the formulation of *B. bassiana* was prepared, a commercial preparation was created, and the importance of its practical use was emphasized. *B. bassiana* is widely used in combination with natural enemies of the potato beetle as well as in formulations used in regular pesticide applications. *B. bassiana* treatments reduce CPB populations by 75% (Cantwell et al., 1980). But the effect is lower compared to chemical insecticides (Campbell et al., 1985; Hajek et al., 1987).

There are commercial preparations with the active ingredient *B. bassiana.* One of them is Mycotrol™. Mycotrol™ is a naturally occurring organism. *B. bassiana* CPB is effective in both larval and adult stages. When B. bassiana is applied, it continues to spread and significantly reduces the population density of CPB throughout the season. Sensitivity to high temperatures It significantly limits the impact of *B. bassiana*. Mycotrol™ is effective at temperatures of 25–30 °C. The development of the organism is slower at higher temperatures. Emerald Bio-Agriculture, produced by M, should be applied when 20–25% of the eggs have hatched.

Figure 2. *a, b, Adults, c) larvae of Leptinotarsa decemlineata applied B. bassiana (Yildirim, K.)*

In recent years, there have been several boipesticides used against CPB, whose active ingredient is *Bacillus thurungiensis* (Bt). "*Bacillus thurungiensis* spp. san diego. is not allowed for use in organic agriculture because it is produced genetically. Novador "*Bacillus thurungiensis* spp. *tenebrionis*" produced by valent USA is not genetically engineered and therefore not allowed for use in organic agriculture. Bt is only effective during the period when it is eaten by pests and during the larval stage. In addition, Bt is generally effective on newly hatched larvae of CPB. Bt treatments should be applied when CPB eggs are at their peak or when 25% of the oldest egg packs have been or are being opened.

It should also be applied when the newly hatched larvae in every 50 plants reach a 70% application density. Bt is most effective when the daily temperature reaches 25 °C and larvae feed intensively (Tingeyet, 1990). Strains of *Bacillus thuringiensis* (Bt) have been sprayed on leaves to control a variety of pests. For many years, foliar sprays containing Bt-based microbial pesticides have been utilized in agriculture; their main active ingredient is cryoproteins. The parasporal crystal protein Cry3A, produced by *Bacillus thuringiensis* var. *tenebrionis* (*B. t. t*.), exhibits insecticidal activities towards CPB. High unit activity and specificity for certain coleopteran insect pests, such as CPB, are characteristics of this protein (Park et al., 2009).

Entomopathogen nematodes

When managing this harmful insect, entomopathogenic nematodes as biological control agents may offer an alternative to chemical pesticides. When tested at the rate of 200–2000 infectious juveniles per individual of CPB in a laboratory bioassay, four species of entomopathogenic nematodes *Steinernema carpocapsae, S. feltiae*, *Heterorhabditis bacteriophora,* and *H. megidis* showed the highest virulence against both larval and adult stages of CPB at temperatures higher than 15 °C. (Trdan et al., 2009). The entomopathogenic nematode *H. marelata* has been shown in another laboratory study to be 100% lethal to CPB larvae (Berry et al., 1997). However, in the field, two applications of this nematode during the potato growing season are only 50% effective in reducing the adult population of CPB (Armer et al., 2004). When managing this harmful insect, entomopathogenic nematodes as biological control agents may offer an alternative to chemical pesticides. When tested

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Plants extracts and Biopesticides

Plant extracts and botanical pesticides fall into two distinct product categories: commercially accessible botanical insecticides and handmade products. Their qualities aren't always suitable for protecting plants, even if they come from nature (Sablon et al., 2013). Apart from hydrolytic molecules or primary metabolites, they are frequently formed by plants in secondary metabolites, frequently

during pest infestations or under adverse environmental conditions. By causing a pest's death or reducing its capacity to reproduce, they function as antifeedants or repellents and aid in the resistance of a variety of pest species. While biopesticides appear to be a viable alternative to traditional chemical insecticides, their practical uses are still restricted, and their research is still in its early stages (Mordue & Nisbet, 2000).

There are commercial plant insecticides used to control CPB. Rotenone is derived from the roots of a plant in South America.Because rotenone kills pests slowly, it is often applied in combination with pyretrum, which has an immediate killing effect. Plant protection takes place in a short time.Rotenone is toxic to fish and pigs and should be used with caution.Since rotenone is a limited substance, it is emphasized that it should be used in cases where other methods are not successful. The use of rotenone is banned by the EU (European Union) (Anonymous, 2023). There are products with different trade names for the control of CPB, with azadirachtin as the active ingredient. These are "Neemix," "Bionem," and "Margosan-0," as they are referred to as. However, it has been reported that it causes phytotoxicity in plants with 1% and higher doses (Olkowski et al., 1992).Additional neem derivatives show side effects similar to the beneficial ones. In a study conducted in Washington, neem products were found to negatively affect ladybirds, especially early larvae (Banken et al., 1998).

Pyola is a natural insecticide combined with canola oil and pyretrum. It is used to control many pests, including aphids, mites, the Mexican bean beetle, and CPB (Anonymous, 2016). However, since most of the canola oil on the market is produced from plants,

this product is not considered organic. Growers should inquire about its certification before using this product. There are many plant species that can be used in the fight against CPB. These include *Nepeta cataria*, *Tanacetum vulgare, Salvia officinalis* (Grossman, 1989), *Cannabis* sp. (Anderman, 2000), *Querqus* sp., *Hedysarum alpinum, and Xanthium strumarium* (Elis et al., 1992; Erdoğan & Toros, 2007). A two-year field study conducted in Canada assessed the effectiveness of spraying plant mixtures as a substitute for conventional CPB management. Bush beans, flax (*Linum usitatissimum* L.), horseradish (*Armoracia rusticana* Gaertn., C.A. Mey. & Scherb.), French marigold (*Tagetes patula* L.), and tansy (*Tanacetum vulgare* L.) were the herbs that were assessed as companions of potato plants (Moreau et al., 2006). Erturk & Sarıkaya (2017) revealed that the extracts of *Liqudambar orientalis* and *Buxus sempervirens* decreased the population of CPB in field conditions and determined that these two plant extracts can be used in the field as a potential alternative to chemical pesticides.

Trap and barrier plants

Trap planting aims to attract CPB to the area where potatoes are grown and remove it from the main crop. For example, in tomato-growing areas where CPB populations are high, potato plants are planted at the edges of the field to collect CPB from the potato plant. Then, before the larvae of the first generation pupate, the potato plant is sprayed to kill the larvae. The plants are then cleared from the field edge (Hoy et al., 1996). This method is not practical or successful when applied to small home gardens. There was no discernible decrease in the number of CPB's in the remaining portion of the field, despite drawing the insects to crops that included larger potato

plants than the rest of the field. Without managing the adults in the trap crop itself, we did not find many opportunities to lower beetle populations using trap crops at the boundaries of potato fields. If growers are ready to monitor and treat the field edges on a regular basis, they can take advantage of naturally occurring adult concentrations near the edges of early and neighboring potato plantings (Hoy et al., 2000). The purpose of CPB, a spring trap crop, is to capture and hold overwintering adult beetles, preventing them from entering the new potato field by placing host plants between the overwintering site and the new field. (French et al., 1993; Weber et al., 1994).

A trap crop should be planted at the edge of a potato field since beetles often overwinter near field borders, especially if the field has been rotated. On the other hand, it has been noted that infestations of potato beetles start at the outside and gradually move inside in both rotated and nonrotated potato fields (French et al., 1993; Weber et al., 1994). Walking overwintered adults frequently leave potato plants after coming into contact with them, which may make it difficult to keep adult beetle concentrations in trap crops (Jermy et al., 1988). It was proposed that blocking this movement between the trap crop and the remaining potato field would prevent the trap crop's seeds from spreading to the remaining area. These barriers need to limit walking speed and increase turning frequency enough to increase the chance of beetles returning to the trap crop in order to increase catch in a trap crop. Small grains or mulch are examples of physical barriers that can hinder beetle dispersal enough to keep them in a trap crop. Chemical barriers may offer more extensive movement discouragement, such as avoiding an area

before it becomes part of the barrier zone. It has been discovered that some pesticides, especially pyrethroids, have repellant properties that work without physical contact and can affect flying insects before they land (Lin et al., 1993).

Numerous insecticides have been ineffective against CPB. Using "trench traps," an inventive method created by AgCanada and studied by Cornell, is a novel way to capture beetles when they leave fields in quest of new food supplies or locations to hibernate. This method, like the majority of effective pest management plans, depends on an understanding of the life cycle of the insect. In an attempt to limit the harm that the potato beetle causes to their plants, farmers frequently rotate their potato crops to adjacent plots of land. By excavating trenches around their fields that are at least 30 cm/12 in. and as deep as 91 cm/3 ft. and lining them with 1.5 mil black plastic mulch, this procedure can be made more effective (Echo, 1995). Since beetles spread by walking at first, crop rotation and/or trenching can greatly minimize infestations. 50% or more of the beetles can be captured in trenches with a slope of 45 degrees or higher (Capinera, 2001). Using row coverings and ditches lined with plastic to act as physical barriers to keep pest insects out of crop fields is another method. To prevent CPBs from getting into the potato crops, synthetic cloth is utilized for that purpose. To prevent most beetles from gaining a firm grip on the surface, the material can be strengthened by adding fine soil particles and arranging them at an angle wider than 46 degrees. After being washed away by rain, a tiny number of beetles may be able to escape, but as soon as the material dries, the protection is restored (Boiteau et al., 1994).

Conclusion

There are many different methods of controlling the potato beetle, including cultural measures, physical and mechanical control, biological control, biopesticides, and chemical control. Today, chemical control is widely practiced to control CPB. However, while some methods, like some IPMs with environmentally friendly pesticides or physical or augmentative control, are already widely accepted and in use, the pure use of conservation biological methods has not yet convinced many farmers, despite the fact that all of these methods have potential. Since alternative approaches and their efficacy are less studied and more complex than most conventional ones, a great deal more research is required. However, it is widely accepted that alternative control strategies make a significant contribution to a healthy environment that benefits all people. This suggests that alternative pest management strategies already have a place in agriculture and will eventually be able to completely replace conventional pesticide-based methods, which are frequently harmful to the environment, or at the very least significantly reduce their use.

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CHAPTER III

Bitki Patojenlerine Karşı Biyokontrol Aktiviteye Sahip Bakterilerin Etki Mekanizmaları

Işıl TEMEL[3]
Mesude Figen YEŞİLDAĞ[4]

Giriş

Ekonominin temel itici güçlerinden biri olan tarım, gelişmekte olan ülkelerin bel kemiğidir. Artan dünya nüfusu göz önüne alındığında gıda arzında da önemli bir artış sağlanması gerekmektedir. Tarım alanlarının kentleşme nedeniyle amaç dışı kullanımından dolayı üretim yapılabilecek alanların azalması artan gıda talebiyle başa çıkmada üreticileri birim alandan daha fazla ürün elde etmeye ve tarım ürünlerinde meydana gelen kayıpları azaltmaya yöneltmiştir (Mauser & ark., 2015; Pradhan & ark., 2015). Zararlı

[3] Dr. Işıl TEMEL, Iğdır Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Iğdır, Türkiye. Orcid: 0000-0001-5968-3609. isil.temel@hotmail.com
[4] Doç. Dr. Mesude Figen YEŞİLDAĞ, Iğdır Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Iğdır, Türkiye. Orcid: 0000-0002-7992-8252. sudefigen@hotmail.com

böcekler, patojenler ve yabancı otlar gibi biyotik etmenler, tarımsal ürünlerde ciddi hasar ve ürün kayıplarına neden olan en önemli bitki zararlılarından bazılarıdır. Niceliksel ve niteliksel gıda üretimini sağlamak için bitkide zarara neden olan bu etmenlerin kontrol altına alınması oldukça önem taşımaktır (Monte, 2001; Heydari & Pessarakli, 2010).

Kimyasal gübre ve pestisit gibi kimyasalların kullanımı, ürün kayıplarını en aza indirmek ve pazarın ekonomik ihtiyaçlarını karşılamak için üreticiler arasında yaygın bir uygulamadır (Benaissa, 2023). Bitki hastalıkları ve zararlılarının etkili bir şekilde kontrol altına alınmasında kimyasal pestisitlerin şüphesiz olumlu katkıları olmasına rağmen, insan sağlığı üzerindeki olumsuz etkilerinin yanı sıra toprak ve suyun kirlenmesi, gıda zincirinde biyolojik birkim ve faydalı organizmalara yönelik olumsuz etkilerine ilişkin endişeler de artmaktadır (Carvalho, 2006; Damalas & Eleftherohorinos, 2011; Geiger & ark., 2010; Kim, Kabir & Jahan, 2017; Negi & ark., 2023). Ayrıca pestisitlerin yoğun kullanımı, dirençli patojenlerin gelişmesine neden olmaktadır (Ash, 2018; Borel, 2017). Bu durum, daha yüksek uygulama dozajlarına ihtiyaç duyulmasına ve farklı etki şekline sahip alternatif pestisitlere olan ilginin artışına yol açmaktadır (Rahman & ark., 2018). Bununla birlikte, bu tür yeni pestisitlerin bulunabilirliği ve çeşitliliği şu anda sınırlı olmakla birlikte artan direnç gelişimi sorununu ortadan kaldırmak için de yetersizdir (Bruce & ark., 2017; Pertot & ark., 2017). Diğer yandan gelişmiş ve gelişmekte olan ülkelerde daha yüksek kaliteli ve sağlıklı gıdalara olan talebin artması (Garcia-Fraile & ark., 2012), bakterilerin, bitki gelişimini teşvik edici ve/veya biyokontrol elemanı olarak kullanılması, kimyasal gübre ve pestisit kullanımını

azaltarak tarım ürünlerinin verim ve kalitesinde artış sağlanmasına imkân vermektedir (Flores-Felix & ark., 2015).

Son yıllarda araştırmacılar, hastalık etmenleriyle mücadelede alternatif bir yöntem olarak potansiyel biyolojik kontrol elemanlarının kullanımına odaklanmıştır (Rahman & ark., 2018). Mikroorganizmalar çevreyi veya hedef olmayan diğer organizmaları tehdit etmeden fitopatojenleri ortadan kaldırabilme özellikleri ile biyokontrol elemanı olarak umut vaat etmektedir. Bu durum sentetik pestisitlerle karşılaştırıldığında, tartışmasız bir avantaj sağlamaktadır (Khamna, Yokota & Lumyong, 2009).

Biyolojik kontrol elemanı olarak bakteriler bitkilerin sağlıklı büyümesi ve gelişmesi için gerekli olan çeşitli moleküllerin üretimi ve dönüşümü yoluyla bitkilere yararlı olmaları, kimyasallara göre daha kısa zamanda daha az maliyetle üretilebilmeleri ve doğadan kolayca elde edilebilmelerinden dolayı organik tarım sistemlerinde de yaygın şekilde kullanılmaktadır (Liu, 2021). Bununla birlikte etkili biyokontrol elemanlarının seçimi için patojen gelişiminin baskılamasının altında yatan mekanizmaların anlaşılması gerekmektedir (Zaim & ark., 2016). Bu konuda son yıllarda bu bakterilerin bitki-mikroorganizma etkileşimlerindeki fizyolojik ve fonksiyonel rollerinin anlaşılmasında önemli ilerleme kaydedilmiş, bakterilerin bitki büyümesini teşvik ettiği mekanizmalar, biyolojik kontrol elemanları olarak hastalığın baskılanmasının altında yatan unsurlar hakkında bilgi sağlanmıştır (Benaissa, 2023). Biyokontrol elemanlarının bitki patojenlerinin baskılanmasında yer alan çok çeşitli mekanizmalar ile hastalık gelişimini azalttığı ve/veya engellediği belirlenmiştir. Bu mekanizmalar arasında siderofor (Temple & ark., 2004), hidrojen siyanid (HCN) (Maurhofer & ark.,

1994; Nandi & ark., 2017), fitohormon (Huang, Shrestha & Huang, 2022), hidrolitik enzim (Gupta & ark. 2015; Benaissa, 2023) ve ACC-deaminaz üretimleri (Safronova & ark., 2006) öne çıkmaktadır.

Biyokontrol Elemanlarının Antagonistik Etki Mekanizmaları

Siderofor Üretimi

Tüm canlı hücreler için önemli mikro elementlerden biri olan demir, fotosentez, solunum, trikarboksilik asit döngüsü, gen regülâsyonu, nitrat sentezi, azot fiksasyonu, ATP ve DNA sentezi gibi biyolojik süreçlerde özellikle kofaktör olarak birçok metabolik işlemlerde düzenleyici rol oynamaktadır (Ratledge & Dover, 2000; Skaar, 2010; Hammer ve Skaar, 2011). Toprakta genelde çözünmemiş olan ferrik demir (Fe^{+3}) formunda bulunan demir canlılar tarafından kullanılamamaktadır. Mikroorganizmaların demir ihtiyaçlarını karşılayabilmek için geliştirdiği demir kazanım mekanizmalarından biri Fe^{+3} şelatlayıcı siderefor molekülleridir. Çeşitli çalışmalar sonucunda sideroforların galyum, aluminyum, bakır, krom, çinko, kurşun, manganez, kadmiyum gibi diğer metalleri de daha düşük afiniteler ile bağlayıp hücre bünyesine aldığı bildirilmiştir (Neilands, 1995; Miller, 2008; Cornelis & Andrews, 2010).

Bakteriler tarafından üretilen sideroforların bitki patojenleri üzerinde de etkili olduğu belirlenmiştir (Vessey, 2003). Biyokontrol bakterileri ve patojenler arasında rekabet yaratan demir bağlayıcı bir protein (Agha & ark., 2023) olan siderofor, demirin (Fe^{+3}) çevreden taşınmasında görevli olan organik bir bileşiktir (Saha & ark., 2016). Ayrıca çeşitli araştırmacılar tarafından sideroforların sistemik kazanılmış direnç (SAR) ve indüklenmiş sistemik dayanıklılık (ISR) elisitörü olarak görev yaptığı da bildirilmiştir (Ongena & ark., 2004;

Ryu & ark., 2004). *Pseudomonas* türlerinin, patates yumrularında *Dickeya* sp.'nin neden olduğu çürümeyi azalttığı, bu durumun siderofor üretimi yoluyla demir iyonları için rekabet etme yeteneğinden kaynaklandığı bildirilmiştir (Czajkowski & ark., 2012). Başka bir çalışmada *Erwinia amylovora*'ya karşı en yüksek antagonistik etkiyi gösteren *Pseudomonas protegens* 59M strainininin başarısında siderofor üretiminin rol oynadığı belirlenmiştir (Mikiciński & ark., 2024). Sureshbabu, Amaresan & Kumar, (2016) tarafından bazı bakterilerin rizosferde bulunan demir iyonlarını fitopatojen funguslar için kullanılamaz hale getirerek gelişimlerini yavaşlatmaya yol açtığı bildirilmiştir. *Pantoea agglomerans*'ın antagonistik etki mekanizmalarını belirlemeyi amaçlayan bir çalışmada *Pantoea aglomerans*'ın demir iyonlarını (Fe+3) bağlayarak bitki direnç mekanizmasını desteklediği rapor edilmiştir (Kramer, Özkaya & Kümmerli, 2020). *Xanthomonas hortorum* pv. *vitians*'e karşı antagonist bakterilerin antibakteriyel aktivitelerinin test edildiği bir çalışmada, patojene karşı en yüksek aktiviteye sahip *Bacillus* strainlerinin (MFD7 ve MFD71) her ikisininde siderofor üretimine sahip olduğu rapor edilmiştir (Dönmez & Temel, 2023).

Hidrojen Siyanid Üretimi

Hidrojen siyanid (HCN) pikomolar konsantrasyonlarda tüm aerobik mikroorganizmalar için oldukça toksik olan bir maddedir. Bakterilerin büyümesine, hareketliliğine ve biyofilm oluşturmasına katkıda bulunan hücre içi ve hücre dışı uçucu bir sinyal molekülü olarak görev yapmakta, sideroforlar ve fenazinler gibi diğer ikincil metabolitlerin üretiminde de rol oynamaktadır. Aynı zamanda, bakterilerin endojen HCN'yi yalnızca kendi hücresel fonksiyonlarını kontrol etmek için kullanmadıklarını, aynı ortamı paylaşan diğer bakterilerin davranışlarını da uzaktan etkileyebildikleri ileri sürülmüştür (Anand & ark., 2020).

HCN biyosentezinin rizosfer bakterileri için oldukça önemli olduğu, HCN sitokrom oksidaz yolunu engelleyerek patojen büyümesini baskıladığı rapor edilmiştir (Zdor, 2014; Nandi & ark., 2017; Sehrawat & ark., 2022). Kök patojenlerinin baskılanmasında bazı floresan Pseudomonaslar tarafından üretilen HCN'nin rol oynadığı, *Thielaviopsis basicola'*nın neden olduğu tütün çürüklüğü hastalığının kontrolünün bu bakteriler tarafından üretilen HCN'den kaynaklandığı tespit edilmiştir (Voisard & ark., 1989). Yapılan çeşitli çalışmalarda HCN gibi antimikrobiyal maddeler üretebilen bakterilerin patojen gelişimini baskılayarak biyokontrol etki gösterdikleri bildirilmiştir (Blumer & Haas, 2000; Duman, 2020; Aşan & ark., 2023). Endofitik bakterilerin fasulye hale yanıklığı hastalığına karşı antibakteriyel aktivitesinin araştırıldığı bir çalışmada hastalık etmenine karşı etkili tüm strainlerin HCN ürettiği bildirilmiştir (Duman & Soylu, 2019).

ACC-Deaminaz Üretimi

Bitki büyümesini kolaylaştırmak ve stresi hafifletmek için bakteriler tarafından kullanılan temel mekanizmalardan bir diğeri ise 1-aminosiklo propan-1-karboksilat (ACC) deaminaz enzimi tarafından bitki etilen seviyelerinin düşürülmesidir (Glick & ark. 2007).

Etilen çok önemli bitki sinyal molekülü olup, tohum çimlenmesi, saçak kök gelişimi, kök nodülasyonu, çiçeklenme ve meyve oluşumu gibi birçok bitkisel fonksiyonda düzenleyici rol oynamaktadır. Ancak, abiyotik (tuzluluk, kuraklık, su baskını, ağır metallere maruz kalma vb.) ve biyotik stres koşullarında (bakteri, virüs, fungus vb.) bitkide bu molekülün üretiminde artış meydana gelmekte ve bu artış bitki gelişimi olumsuz etkilemektedir (Yang,

Kloepper & Ryu, 2008). Bitkilerde etilen oluşumunu hidrolize etmek için ACC deaminaz (1- aminosiklopropan-1-karboksilat deaminaz) üretebilen bazı bakteriler bitkide etilen seviyelerini düzenleyerek yüksek etilenin olumsuz etkilerini azaltmaktadır (Gamalero & Glick, 2015). Kardaş & Ökmen, (2014) tarafından yapılan çalışmada ACC-deaminaz üreten bakterilerin çeşitli biyotik ve abiyotik stres faktörlerinin neden olduğu zararı azaltmada önemli bir etkiye sahip olduğu ortaya konulmuştur. Gamalero & Glick (2015) tarafından yapılan bir çalışmada, ACC deaminaz üreten *Pseudomonas* straininin, bitkide fitoplazmaların enfeksiyonundan kaynaklanan stresin azaltılmasında etkili olduğu bildirmiştir.

Fitohormon Üretimi

Bitki hormonları olarak bilinen küçük moleküllü maddeler, stres ve büyüme tepkileri boyunca çok sayıda fizyolojik işlevde önemli bir rol oynamaktadır (Ma & Ma 2016). Birçok bakteri straini bitki gelişiminin farklı aşamalarında yer alan organik moleküller olarak tanımlanan fitohormonları sentezleyebilmektedir (Sokolova & ark., 2011). Bazı mikroorganizmalar tarafından sentezlenen fitohormonlar, bitki patojenezinde rol oynayabilmekte, bitki büyüme ve gelişmesine katkıda bulunmaktadır (Spaepen, 2015). Absisik asit, oksinler, sitokininler, etilen ve gibberellinler en yaygın fitohormonlar arasında yer almaktadır. Brassinosteroid, oligosakkaritler, biyoaminler, salisilatlar-salisilik asit ve jasmonik asit tanımlanan birkaç yeni fitohormon veya hormon benzeri bileşiktir (Tsavkelova & ark. 2006). İndol asetik asit (IAA) bitki büyüme ve gelişme düzenleyicisi olarak iyi bilinmesine rağmen, bitki ve mikroorganizma arasındaki etkileşimlerde rol oynayarak patojenlerin neden olduğu enfeksiyona karşı bitkinin direncini

etkilemektedir (Kunkel & Harper 2018). Antraknoz hastalığının biyokontrolü ve daha iyi sorgum bitkisi gelişimi, *Trichoderma harzianum*'un IAA üretimiyle ilişkilendirilmiştir (Saber & ark., 2017). Bu nedenle, fitohormonların mekanizmasının ve etkisinin daha iyi anlaşılması, patojenlerin yönetimi için yeni stratejilerin ortaya çıkarılmasına yardımcı olacaktır.

Hidrolitik Enzim Üretimi

Kitinaz

Kitin, kitinazlar tarafından hidrolize edilen N-asetilglukozaminin (GlcNAc) çözünmeyen doğrusal bir homopolimeridir (Berini & ark., 2018). Kitini oluşturan N-asetilglukozamin polimeri, gezegendeki en yaygın ikinci polisakkarittir ve β-(1,4) bağları ile bağlanmaktadır (Adrangi & Faramarzi, 2013). Doğada yaygın olarak bulunmakta ve eklembacaklıların dış iskeletinde, mantarların hücre duvarında, kabukluların kabuklarında ve nematodların kütikülasında işlevsel polisakkarit olarak görev yapmaktadır. Kitin, α-, β- ve γ-kitinler olmak üzere üç polimorfik formda bulunmaktadır (Dahiya, Tewari & Hoondal, 2006). Sindirim, hücre farklılaşması ve kütikül dönüşümünde spesifik rolleri olan geniş kitinaz aileleri bakterilerde, bitkilerde ve böceklerde test edilmiştir. Bununla birlikte, birçok hayvan, bitki ve böcek, kitinazlara benzeyen ancak katalitik bölge içermeyen lektinler üreten genleri de ifade etmektedir (Arakane & Muthukrishnan 2010).

Aspergillus terreus'un saflaştırılmış kitinolitik enziminin *A. niger*, *A. oryzae*, *Penicillium oxysporium*, *Rhizoctonia solani*, *Candida albicans* ve *Fusarium solani*'nin büyümesini inhibe etme

yeteneğine sahip olduğu bildirilmiştir (Farag & ark., 2016). Brzezinska, Jankiewicz & Walczak, (2013) tarafından yapılan çalışmada, *Streptomyces rimosus'*dan saflaştırılmış kitinazın *in vitro* ortamda *Fusarium solani* ve *Alternaria alternata*'ya karşı antifungal aktivite gösterdiği saptanmıştır. Yüksek kitinaz üreticisi olan *Paenibacillus* sp D1'nin, *Helicoverpa armigera*'nın kontrolünde etkili olduğu, bu strainin larvalarda %40 ölüm oranına neden olduğu görülmüştür (Singh, Singh & Joshi, 2016). Kitinaz ve β-glukonaz üreten bir *Pseudomonas* strainin, pekçok tarımsal ürünün en tahripkâr patojenlerinden olan *Phytophthora capsici* ve *Rhizoctonia solani*'nin sebep olduğu hastalıkları engellemede başarılı bulunduğu bildirilmiştir (Maksimov & ark., 2011).

Selülaz

Bitki biyokütlesinin %40'ını oluşturan selülaz yaklaşık 15000 glikoz biriminin ß-1,4- glikozidik bağlar ile linear bir şekilde bağlanması ile meydana gelmektedir. Suya karşı yüksek çekiciliği olmasına rağmen, suda çözünmemektedir. En az üç farklı enzimin sinerjistik etkisi ile glikoza hidrolize olabilmektedir. Bu enzimler; ekzoglukanaz, endoglukanaz ve ß-glukosidaz'dır (Niehaus & ark., 1999). Bu enzimler geniş ölçüde fungus ve bakterilerden elde edilmekte ve çeşitli biyoteknolojik uygulamalarda kullanılmaktadır. Ticari olarak en çok kullanılan selülaz *Trichderma* sp. (Teeri & ark, 1998) tarafından üretilmektedir. Ayrıca selülazlar *Bacillus, Aspergillus* ve *Penicillium* strainlerinden de elde edilmektedir (Tomme & ark., 1995; Ito, 1997).

Antibiyotik üreten *Streptomyces* ve sellülaz üreten *Micromonospora'*dan oluşan kombinasyonun, *Phytophthora cinnamomi*'nin sebep olduğu kök çürüklüğünü baskıladığı rapor

edilmiştir (Kumar & ark., 2010). Farklı bir çalışmada, *Micromonospora* sp.'nin, bitki probiyotiği olduğu *ve Botrytis cinerea* enfeksiyonu sonucu domates bitkilerinde sistemik direnci indüklediği bildirilmiştir (Martínez-Hidalgo, García & Pozo, 2015). Sellülaz üreten *Streptomycetes* strainlerinin hıyarda *Pythium aphanidermatum*'un gelişiminin engellenmesinde etkili olduğu bildirilmiştir (Kumar & ark., 2010).

Proteaz

Proteazlar, proteinleri daha küçük peptit zincirlerine ve amino asit gruplarına parçalayan hidrolitik enzimlerdir. Substratın amino veya karboksi terminallerine yakın peptid bağlarını parçalayan ekzopeptidazlar ve substratın terminallerinden uzakta peptid bağlarını parçalayan endopeptidazlar, proteazların yaygın olarak kategorize edildiği iki ana kategoridir (Rao & ark., 1998). Yaşam için fizyolojik olarak gerekli olan ve mikroorganizmalar, hayvanlar ve bitkiler de dahil olmak üzere çeşitli kaynaklarda bulunabilen bu enzimler, kelimenin tam anlamıyla tüm hücresel süreçlerde çok sayıda rol oynamaktadır. Proteaz enzimleri, doğada hayvansal, bitkisel ve mikrobiyal atıkların parçalanmasında önemli rol oynayarak besin döngüsünde yer almakta ve ayrıca bitkilerin besin alımlarını da artırmaktadır (Yadav & ark. 2016).

Biyoteknolojik uygulamalar için istenen özellikler olan metabolik çeşitlilik, hızlı gelişim ve genetik modifikasyona duyarlılık nedeniyle mikroorganizmalar ideal bir proteaz kaynağı oluşturmaktadır (Khan & Ahmad 2019). Proteaz patojenin hücre duvarındaki glikoprotein bağını parçalamak suretiyle patojen gelişimini inhibe etmektedir (Banani & ark., 2014). Topraktan izole edilmiş *Saccharomonospora viridis* tarafından salınan hücre dışı

kütikül parçalayıcı bir proteaz*, Panagrellus redivivus*'a karşı güçlü nematotoksik etki göstermektedir (Darwesh & ark. 2019). Farklı bir çalışmada proteolitik aktiviteye sahip bakterilerin, proteaz enzimi kullanarak hastalık etmenlerinin protein yapısını bozarak patojenlerin gelişimini engellediği bildirilmiştir (Santoyo & ark., 2021).

Antagonist bakterilerin etki mekanizmalarının bilinmesi, bitki patojenleriyle mücadelede kullanılacak etmenin seçiminde kolaylık sağlamakta, daha hızlı ve etkili yöntemin belirlenmesine imkân tanımaktadır. Bu bağlamda mevcut çalışmada, farklı bitki patojenlerine karşı antibakteriyel aktivitesi belirlenen bakteri strainlerinin bazı *in vitro* antagonistik etki mekanizmalarının belirlenmesi amaçlanmıştır.

Yöntem

Antibakteriyel Aktiviteye Sahip Bakteri Strainleri

Farklı çalışmalarda çeşitli bitki patojenlerine karşı antibakteriyel etki gösteren bakteri strainlerinleri hakkında detaylı bilgi Çizelge 1'de sunulmuştur.

Çizelge 1. Antibakteriyel aktiviteye sahip bakteri strainleri ve etkili oldukları patojenler

Strain No	MIS* Tanı Sonucu	Antibakteriyel etki gösterdiği patojen	Literatür
ZA25	*Bacillus megaterium*	*Clavibacter michiganensis* subsp. *michiganensis*	Kurt, 2023
Ö7	*Bacillus cereus*		
ÖBF76	*Pseudomonas putida*		
ÖSLM3/6	*Bacillus pumilus*		
ÖSLM5/6A	*Brevibacillus parabrevis*		
ÖSLM7/6A	*Brevibacillus parabrevis*		
ÖSLM8/9B	*Kocuria rosea*		
ÖSLM 3/7	*Bacillus alcalophilus*	*C.m.* subsp. *michiganensis, Pseudomonas syringae pv. phaseolicola,*	Kurt, 2023 Dönmez, & Aliyeva, 2021
ZA79	*Pseudomonas fluorescens*	*Pseudomonas savastanoi* pv. *phaseolicola, Xanthomonas axanopodis* pv. *phaseoli*	Dönmez, & Aliyeva,
ZA114	*Bacillus subtilis*		
ZA142	*Bacillus atrophaeus*		
MFY7	*Bacillus subtilis*	*Xanthomonas axanopodis* pv. *vesicatoria*	Almast, 2023
MFY12	*Bacillus amyloliquefaciens*		
MFY25	*Pseudomonas putida*		
MFY34	*Bacillus licheniformis*		
MFY42	*Bacillus pumilus*		
MFY98	*Bacillus subtilis*		
MFY101	*Brevibacillus brevis*		
MFY404	*Bacillus subtilis*		

*MIS: Mikrobial Identification System

Bakterilerin Antibakteriyel Etki Mekanizmalarının Belirlenmesi

Hidrojen Siyanid (HCN) Üretimi

Antibakteriyel aktivitesi belirlenen strainlerin HCN üretimi kalitatif olarak belirlenmiştir. Bu amaçla bakteri strainleri, King B besi yerine ekilmiştir. Ardından ekim yapılan petri kabının kapağına

yerleştirilen filtre kağıtları yaklaşık 1 ml pikrik asit çözeltisi (2,5 g pikrik asit ve 12,5 g Na_2CO_3 1000 ml sdH_2O) ile nemlendirilmiş ve petrinin etrafı parafilm ile kaplanmıştır. Petriler 27°C'de 48 saat inkübasyona bırakılmıştır. Filtre kâğıdının renginin sarıdan kahverengiye dönüşmesi pozitif sonuç olarak değerlendirilmiştir (Bakker & Schippers, 1987).

ACC-Deaminaz Üretimi

Bakteri strainlerinin ACC-deaminaz üretimi DF (Dworkin and Foster) Salt besi yerinde test edilmiştir. 2 g $NaHPO_4$, 1,33 g KH_2PO_4, 0,67 g glukoz, 0,067 g Mg $SO_4 7H_2O$, 0,67 g sitrik asit, 0,67 g glukonik asit, 41,53 mg $ZnSO_4 7H_2O$, 0,34 mg $FeSO_4 7H_2O$, 3,33 mg H_3BO_3, 3,73 mg $MnSO_4 H_2O$, 26,07 mg $CuSO_4 5H_2O$ ve 3,33 mg MoO_3 300 ml sdH_2O içerisine ilave edilerek magnetik karıştırıcıda homojen bir hal alıncaya kadar karıştırılmıştır. Karışımın pH'sı 7.2' ye ayarlandıktan sonra agar ilave edilmiş ve 121°C'de 15 dk otoklav edildikten sonra 45°C' ye kadar soğutulmuştur. 30 ml steril distile su içerisinde 100 mg ACC çözdürülüp 0,45 µl' lik filtreden geçirilerek besi ortamına ilave edilerek hazırlanmıştır. Bakteri strainleri besi ortamına çizgi ekim metodu ile ekilmiş, petriler 27°C'de 48-72 saat inkübe edilerek koloni gelişimleri gözlemlenmiştir. Besi ortamında gelişim gösteren strainler ACC-deaminaz pozitif olarak kabul edilmiştir (Penrose & Glick, 2003).

Siderofor Üretimi

Bakterilerin siderofor üretimi CAS agar besi yerinde test edilmiştir. Besi yerinin aşağıda belirtildiği şekilde hazırlanmıştır.

A) CAS Solüsyonu: 1) 0,06 g CAS 50 ml sdH_2O içerisinde çözdürülmüştür. 2) 0, 0027 g $FeCl_3 6H_2O$ 10 ml HCI (10Mm)

içerisinde çözdürülmüştür (100 ml saf su içerisine 83 µl HCI ilave edilmiş ve buradan 10 ml alınmıştır). 3) 0,073 g HDTMA 40 ml saf su içerisinde çözdürülmüştür. Bu üç solüsyon karıştırılıp otoklavda steril edilmiştir.

B) Minimal tuz ortamı (MM9): 1) Salt stok solüsyonu; 15 g KH_2PO_4, 25 g NaCI, 50 g NH_4CI 500 ml saf su içerisinde çözdürülmüştür. 2) %20 glukoz stok solüsyonu; 20 g glukoz 100 ml saf su içinde çözülmüştür. 3) NaOH stok solüsyonu; 25 g NaOH 150 ml saf su içerisinde çözdürülerek (pH~ 12) ortamın pH' sını ayarlarken kullanılmıştır.

C) Casamino asit solüsyonu: 4 g casamino asit 36 ml saf su içerisinde çözdürülmüştür. İz miktarda demiri kaldırmak için, %3' lük 8-hydroxyquinoline kloroform içerisinde çözdürülmüştür (1,08g/36 ml). Karışım casamino asit solüsyonuna eklenip 24 saat bekletilmiştir (Besi yerine katarken 0,22 µl' lik filtreden geçirilerek steril edilmiştir).

D) CAS agar hazırlama: 750 ml saf su içerisine 100 ml salt stok solüsyonundan eklenmiştir. Karışımın pH' sı 6' ya ayarlandıktan sonra 32,24 g Pipes (piperazine-N, N'-bis2-ethanesulfonic acid) yavaş yavaş eklenmiştir (Pipes pH 5' in altında çözülmez). Rengin yeşile dönmemesi için pH' nın 6,8' i geçmemesine dikkat edilmiştir. Pipes tamamen çözüldükten sonra 15 g bakteriyolojik agar ilave edilerek ortam otoklav edilmiştir.

Steril kabin içerisinde D solüsyonuna 30 ml steril casamino asit solüsyonu konulmuş ve üzerine 10 ml steril %20' lik glukoz solüsyonundan ilave edilmiştir. Daha sonra 100 ml A solüsyonu yavaşça ortama eklenmiş ve petrilere dökülerek soğutulmuştur.

Antagonist bakteri strainleri çizgi ekimle hazırlanan besi yerine inokule edilmiş ve inkübasyona (27°C'de 5 gün) bırakılmıştır. Bakteri kolonisinin etrafında portakal renkli zon oluşması siderofor pozitif olarak değerlendirilmiş ve bu alanın çapı (mm) ölçülmüştür (Louden & ark., 2011).

Kitinaz Üretimi

Bakterilerin kitinaz üretimi Kitin Agar besi yerinde test edilmiştir. Bu amaçla 10 g kitin, 100 ml %37' lik HCl içerisine ilave edilmiş ve oda sıcaklığında 2 saat veya kitin tamamen eriyene kadar magnetik karıştırıcıda karıştırılmıştır. Kitin tamamen eriyince içerisine 500 ml soğuk etanol yavaş yavaş ilave edilerek solüsyon çökeltilmiştir. Daha sonra süspansiyonun pH' sı 10 N NaOH ile nötralize edilmiştir. Ardından süspansiyon 8000 rpm' de 10 dakika santrifüj edilmiş ve çökelti besi yerine ilaveye hazır hale getirilmiştir. 6 g Na_2HPO_4, 3 g KH_2PO_4, 1 g NH_4CI, 0,5 g NaCI, 0,05 g yeast extract ve 15 g agar ve hazırlanan kitin 1 litre sdH_2O içerisine ilave edilmiştir. Hazırlanan karışım otoklav edildikten sonra 45˚C' ye soğutulup petrilere dökülmüştür. Kitin agar besi yerine bakterilerin nokta ekimi yapılmış ve 30˚C' de 72 saatlik inkübasyondan sonra kolonilerin çevresinde oluşan şeffaf zon pozitif sonuç olarak değerlendirilmiştir (Faramarzi & ark. 2009).

Selülaz Üretimi

Bakteri strainlerinin selülaz aktivitesi CMC Agar besi yerinde belirlenmiştir. Dört ayrı solüsyondan oluşan besi yeri aşağıda belirtilen şekilde hazırlanmıştır.

- Solüsyon A: 0,25 g NaCl, 1,5 g K_2HPO_4, 2,5 g karboksi metil selüloz (CMC) 400 ml deiyonize su

içerisine konulmuştur. Topaklaşmayı engellemek için CMC ortama yavaş yavaş eklenmiş ve 15 dakika manyetik karıştırıcıda karıştırılmıştır.

- Solüsyon B: 3 g Na_2HPO_4, 0,5 g NH_4Cl, 2,5 g glycerol, 0,5 g yeast ekstrakt, 6,5 g bakteriyolojik agar 100 ml deiyonize su içerisine ilave edilmiştir.
- Solüsyon C: 1 M $MgSO_4$.
- Solüsyon D: %7,5 v/w $CaCl_2$.

Her bir solüsyon ayrı ayrı otoklavda steril edilmiş, solüsyon A ve B sıcakken karıştırılmış, bu karışım üzerine solüsyon C ve D den 1'er ml eklenmiştir. Köpük oluşmasına izin verilmeden dikkatlice karıştırılan besi ortamı petrilere dökülmüştür. Petriler 24-48 saatlik Nutrient Agar (NA) besi ortamında gelişen bakteri kültürleri ile inokule edilmiş ve 27°C'de 4 gün inkubasyona bırakılmıştır. İnkubasyonun ardından petri yüzeyi 10 ml %0,1 kongo kırmızısı solüsyonu (0,1 g kongo kırmızısı, 100 ml sdH_2O) ile kaplanmış ve 20 dakika bekletilmiştir. Bu sürenin ardından petrilerden solüsyon dökülerek uzaklaştırılmış ve petri yüzeyi 10 ml 1 M NaCl ile kaplanarak 5 dakika bekletilmiştir. Kırmızı renk alan besi ortamında kolonilerin etrafında sarı renkli zon oluşumu pozitif sonuç olarak değerlendirilmiş ve zonun çapı (mm) ölçülerek kaydedilmiştir (Klement & ark., 1990).

Proteaz üretimi

Bakterilerin proteaz üretimlerini belirlemek amacıyla Skim Milk Agar (SMA) besi yeri kullanılmıştır. 15 g skim milk, 0,5 g yeast ekstrakt ve 9,3 g agar 1 L sdH_2O içerisine ilave edilmiştir. Ortam

110°C' de 10 dk steril edilmiş ve 45°C' ye soğutulduktan sonra petrilere dökülmüştür (Besi ortamının hazırlanmasında kullanılan erlen ve su önceden 121°C' de 15 dk steril edilmiştir). Bakteri strainlerinin 24 saatlik kültürlerinden alınan koloniler hazırlanan besi ortamına nokta şeklinde ekilmiş ve petriler 5 gün boyunca inkübe edilmiştir. Bakteri kolonisi çevresinde oluşan şeffaf zon pozitif sonuç olarak değerlendirilmiş ve zon çapı (mm) ölçülerek kaydedilmiştir (Ullah & ark. 2017).

Bakteri Strainlerinin Fitohormon Üretimi

In vitro ikili testlerde biyokontrol etkinliği belirlenen antagonist bakteri strainlerinin fitohormon ve salisilik asit üretimi, High Liquid Pressure Chromatography (HPLC) cihazı kullanılarak kalitatif ve kantitatif olarak belirlenmiştir. Hormon ekstraksiyonu için NB besi ortamında geliştirilen bakterilerden hazırlanan solüsyondan 5 ml alınarak falkon tüplere konulmuştur. Bakteri solusyonunun üzerine -40°C'de bekletilen %80'lik metanolden 15 ml (HPLC grade) ilave edilmiş ve 6000 rpm' de10 dk ultra doku parçalayıcı (Ultrasonic Processor Jenway Ltd.) içerisinde homojenize edildikten sonra karanlıkta +4°C'de 24 saat bekletilmiştir. Ardından örnekler 10000 rpm' de ve 4°C'de 30 dk homojenize edilmiş ve filtre kâğıdından (Whatman No:1) süzülmüştür. Elde edilen filtratlar, PTFE (Politetra-Floretilen; 0.45 µm) filtrelerinden ve Sep-Pak C18 (Waters) kartuşlarından geçirilmiş ve %80 metanol ile çözdürülerek HPLC cihazında analiz edilmek üzere 2 ml' lik viallere konularak, -20°C'de muhafaza edilmiştir (Şekil 3.8). Cihazda okuma yapılırken C18 (4,6 x250 mm, 5µm) kolonu, mobil faz olarak ise asetonitril ve %1'lik fosforik asit kullanılmıştır. Numunenin kolondan akış hızı 0.6 ml/da ve kolon

sıcaklığı 25°C'ye ayarlanmıştır. Okuma 265 ve 310 nm dalga boylarında yapılmıştır (Atıcı, Ağar, & Battal, 2003).

Sonuç

Bakteri strainlerinin antibakteriyel etki mekanizmalarını belirlemek amacıyla yapılan testler sonucunda strainlerin sahip olduğu etki mekanizmaları belirlenmiş ve bütün strainlerin en az bir etki mekanizmasına sahip olduğu bulunmuştur. Strainlerin fitohormon üretimlerine ait sonuçlar Çizelge 1'de, ACC-deaminaz, siderofor, hidrojen siyanid üretimleri ise Çizelge 2'de sunulmuştur. Bakteri strainlerinin litik enzim üretimlerine ait sonuçlar ise Çizelge 3'te verilmiştir.

Çizelge 1. Test edilen bakterilerin fitohormon (IAA; Zeatin, Giberallik asit) üretimleri (ng/µl)

Strainler	MIS Tanı Sonucu*	Zeatin	GA	İAA
Ö7	*Bacillus cereus*	-	-	-
ÖBF76	*Pseudomonas putida*	14,8	45,1	-
ÖSLM 3/6	*Bacillus pumilus*	20,1	-	4,9
ÖBF76	*Brevibacillus parabrevis*	37,47	-	-
ÖSLM7/6A	*Brevibacillus parabrevis*	25,7	-	-
ÖSLM8/9B	*Kocuria rosea*	-	-	-
ÖSLM 3/7	*Bacillus alcalophilus*	29,1	-	4,38
ZA25	*Bacillus megaterium*	-	-	-
ZA79	*Pseudomonas fluorescens*	25,2	86,2	-
ZA114	*Bacillus subtilis*	22,5	-	-
ZA142	*Bacillus atrophaeus*	26,4	-	-
MFY7	*Bacillus subtilis*	18,7	24,1	-
MFY12	*Bacillus amyloliquefaciens*	4,7	15,77	-
MFY25	*Pseudomonas putida*	9,8	-	3,7
MFY34	*Bacillus licheniformis*	19,6	-	-
MFY42	*Bacillus pumilus*	14,1	-	-
MFY98	*Bacillus subtilis*	23,9	-	-
MFY101	*Brevibacillus brevis*	6,1	28,5	-
MFY404	*Bacillus subtilis*	16,4	131,7	-

*MIS: Mikrobial Identification System, GA: Giberellik asit, İAA: İndol asetik asit

Çizelge 1'de verilen sonuçlar incelendiğinde strainler arasında en yüksek zeatin üretimi *Brevibacillus parabrevis* strain ÖSLM 5/6A'da, en yüksek giberellik asit üretimi *Pseudomonas putida* strain ÖBF76'da ve en yüksek indol asetik asit üretimi ise *Bacillus pumilus* strain ÖSLM 3/6'da tespit edilmiştir. Test edilen strainlerden üç strain hariç tamamında zeatin üretimi belirlenirken sadece ÖBF76, ÖSLM 3/7 ve MFY25 strainlerinde indol asetik asit üretimi tespit edilmiştir. Salisilik asit ve absisik asit üretimi strainlerin hiçbirinde belirlenememiştir (Çizelge 1).

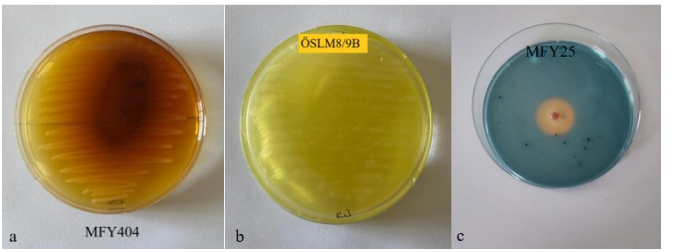
Çizelge 2. *Test edilen bakterilerin ACC-deaminaz, siderofor ve hidrojen siyanid üretimleri*

Strainleri	MIS Tanı Sonucu	ACC-deaminaz	Siderofor (mm)	HCN
Ö7	*Bacillus cereus*	-	15	-
ÖBF76	*Pseudomonas putida*	-	-	-
ÖSLM 3/6	*Bacillus pumilus*	K+	11	-
ÖSLM 5/6A	*Brevibacillus parabrevis*	+	10	-
ÖSLM 7/6A	*Brevibacillus parabrevis*	+	7	-
ÖSLM8/9B	*Kocuria rosea*	+	8	-
ÖSLM 3/7	*Bacillus alcalophilus*	+	10	-
ZA25	*Bacillus megaterium*	K+	10	-
ZA79	*Pseudomonas fluorescens*	-	10	-
ZA114	*Bacillus subtilis*	K+	15	-
ZA142	*Bacillus atrophaeus*	+	7	-
MFY7	*Bacillus subtilis*	+	20	-
MFY12	*Bacillus amyloliquefaciens*	-	5	-
MFY25	*Pseudomonas putida*	-	34	-
MFY34	*Bacillus licheniformis*	K+	7	-
MFY42	*Bacillus pumilus*	-	-	-
MFY98	*Bacillus subtilis*	+	26	-
MFY101	*Brevibacillus brevis*	-	7	-
MFY404	*Bacillus subtilis*	+	-	+

*MIS: Mikrobial Identification System, K+: Kuvvetli pozitif, +: Pozitif, -: Negatif

Çizelge 2'de yer alan değerler incelendiğinde, *Bacillus pumilus* ÖSLM 3/6, *Bacillus megaterium* ZA25, *Bacillus subtilis*

ZA114 ve *Bacillus licheniformis* MFY34 strainlerinin ACC-deaminaz test sonucunun kuvvetli pozitif olduğu görülmüştür. Test edilen strainler arasında en yüksek siderofor üretimi *Pseudomonas putida* strain MFY25'te tespit edilirken, HCN üretimi strainlerden sadece *Bacillus subtilis* strain MFY404'te belirlenmiştir (Şekil 1).



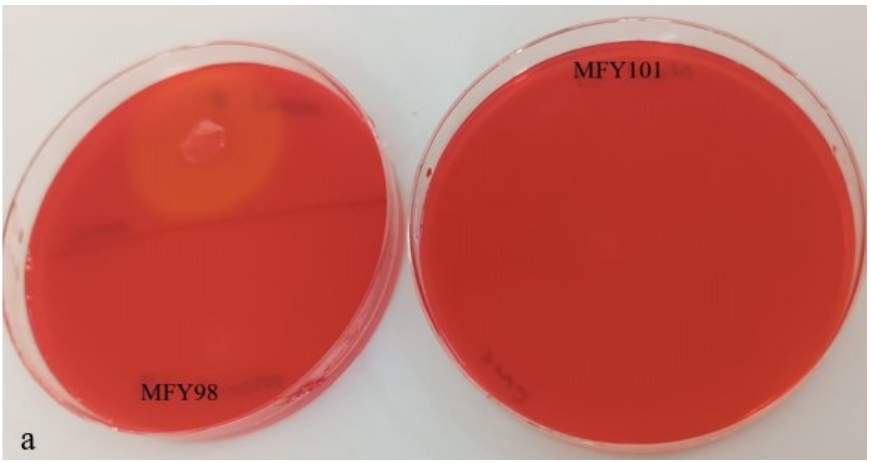
Şekil 1.Hidrojen siyanid üretimi pozitif (a), hidrojen siyanid üretimi negatif (b) ve siderofor üretimi pozitif (c) olan bakteri strainleri

Çizelge 3. Test edilen bakterilerin hidrolitik enzim üretimlerinin belirlenmesi

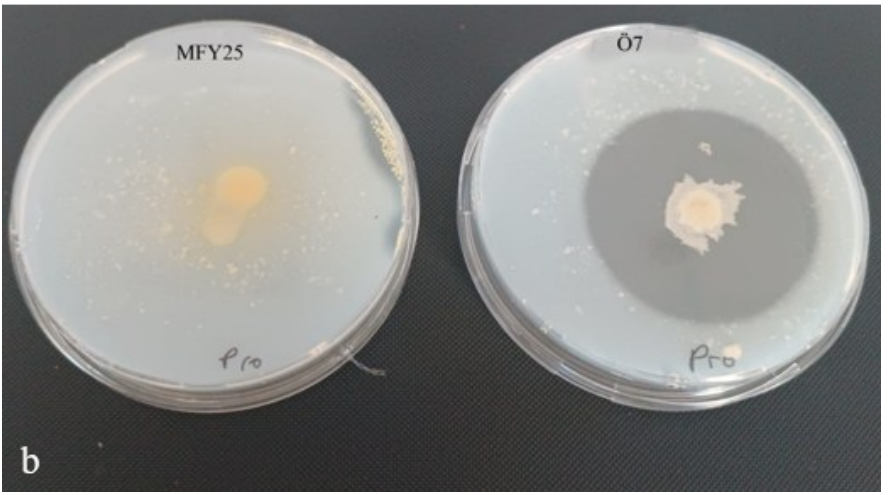
Strainler	MIS Tanı Sonucu	Selülaz (mm)	Proteaz (mm)	Kitinaz (mm)
Ö7	*Bacillus cereus*	20	65	-
ÖBF76	*Pseudomonas putida*	21	40	-
ÖSLM3/6	*Bacillus pumilus*	-	30	-
ÖSLM5/6A	*Brevibacillus parabrevis*	20	45	-
ÖSLM7/6A	*Brevibacillus parabrevis*	25	38	-
ÖSLM8/9B	*Kocuria rosea*	24	31	-
ÖSLM3/7	*Bacillus alcalophilus*	22	35	-
ZA25	*Bacillus megaterium*	25	40	-
ZA79	*Pseudomonas fluorescens*	-	53	-
ZA114	*Bacillus subtilis*	-	36	-
ZA142	*Bacillus atrophaeus*	-	38	-
MFY7	*Bacillus subtilis*	30	24	-
MFY12	*Bacillus amyloliquefaciens*	-	39	-
MFY25	*Pseudomonas putida*	-	-	-
MFY34	*Bacillus licheniformis*	22	80	-
MFY42	*Bacillus pumilus*	21	37	-
MFY98	*Bacillus subtilis*	24	29	-
MFY101	*Brevibacillus brevis*	-	48	-
MFY404	*Bacillus subtilis*	20	38	-

*MIS: Mikrobial Identification System

Çizelge 3 değerlendirildiğinde, 12 strainin selülaz aktivitesine sahip olduğu ve en yüksek selülaz üretiminin *Bacillus subtilis* strain MFY7'den elde edildiği belirlenmiştir (Şekil 2). Yedi strainde ise aktivite tespit edilememiştir. Strainlerin proteaz aktiviteleri incelendiğinde *Bacillus licheniformis* strain MFY25 hariç test edilen tüm strainlerin proteaz üretebildiği, en yüksek proteaz üretiminin ise *Bacillus licheniformis* MFY34 strainınden elde edildiği belirlenmiştir (Şekil 3). Ancak bütün strainlerin kitinaz aktivitesinin negatif olduğu görülmüştür.



Şekil 2. Selülaz aktivitesi pozitif (solda) ve negatif (sağda) olan strainler



Şekil 3. Proteaz aktivitesi negatif (solda) ve pozitif (sağda) olan strainler

Çalışmadan elde edilen sonuçlar her bir strainin en az bir etki mekanizmasına sahip olduğunu, bazı strainlerin ise birden çok mekanizma ile etki ettiğini göstermiştir. Bu strainlerin sahip olduğu etki mekanizmalarının bilinmesi farklı patojenlerin kontrolü amacıyla yürütülen çalışmalarda kullanılmak üzere seçimlerinde ve hastalık etmenlerine karşı daha etkili mücadele yönteminin belirlenmesinde zamandan ve malzemeden tasarruf sağlanmasına katkı sağlayacaktır.

Bitki hastalıkları, tarım ürünlerinin verim ve kalitesinde kayıplara yol açan unsurların başında gelmektedir. Üreticiler meydana gelen bu zararı engellemek veya ekonomik zarar eşiğinin altında tutmak için yoğun pestisit kullanımına yönelmiştir. Bunun sonucu olarak da doğal kaynaklarda geri döndürülemeyen kayıplar meydana gelmeye başlamıştır. Ayrıca bu kimyasalların insan sağlığına olan olumsuz etkileri de gün geçtikçe artmaktadır. Bitki

hastalıklarının kontrolünde antimikrobiyal etki mekanizmalarına sahip faydalı bakterilerin kullanımı, şüphesiz doğal kaynakların korunmasına ve tarımsal üretimde sürdürülebilirliğin sağlanmasına önemli katkılar sunacaktır. ACC-deaminaz pozitif olan strainlerin tarımda kullanımı biyotik ve abiyotik stres altında bitkinin normal gelişimini sürdürmesine yardımcı olurken, çeşitli hidrolitik enzim üretimine sahip olan strainler farklı patojenlerin gelişimini sınırlayarak bitkilerin hastalıklara karşı korunmasını sağlayacaktır. Mekanizmaları tespit edilen strainlerin çoğunun ekstrem koşullarda hayatta kalabilen, endospor üreten *Bacillus* türleri olması ise biyokontrol aktivitesini başarılı kılacaktır.

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