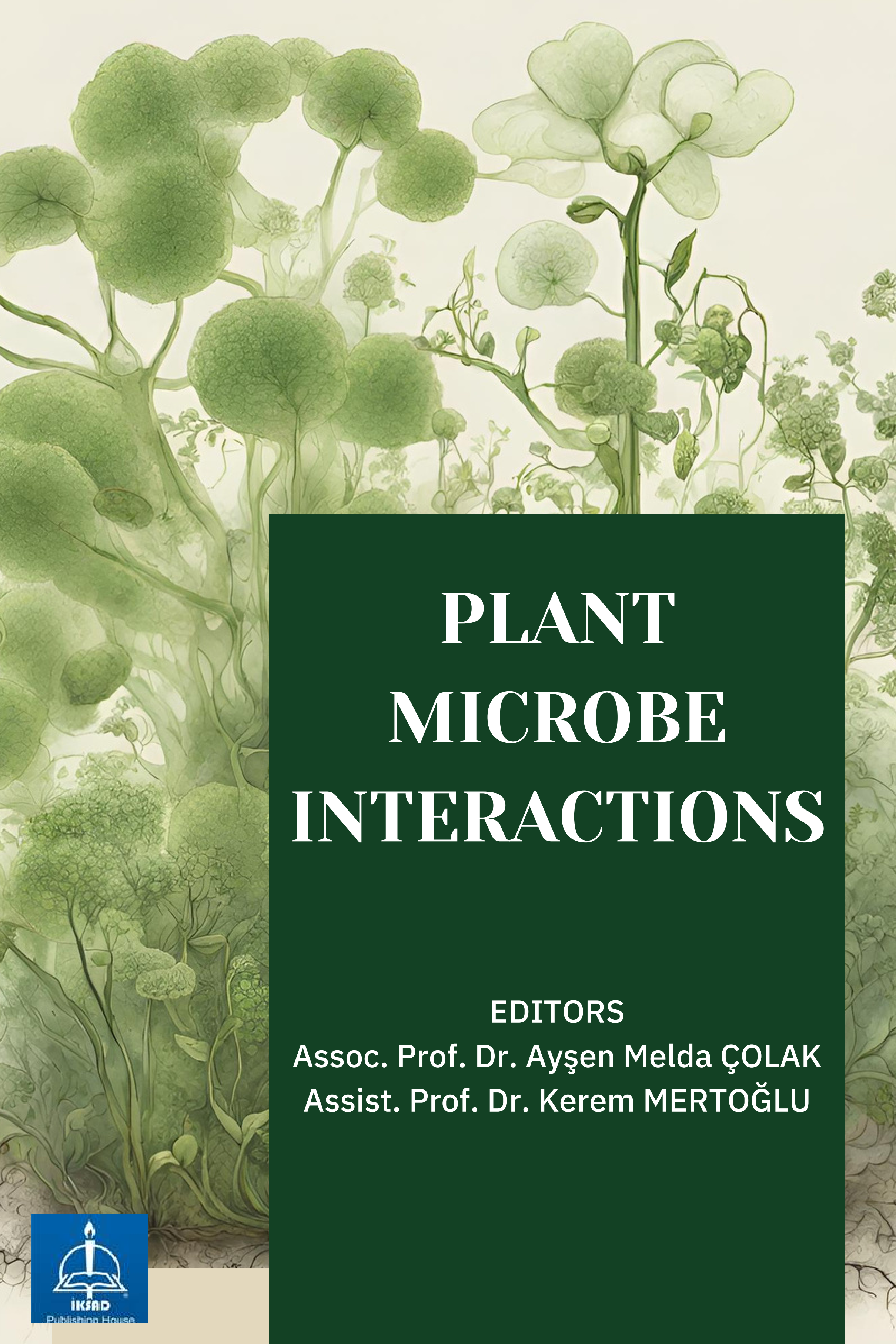


PLANT MICROBE INTERACTIONS

EDITORS
Assoc. Prof. Dr. Ayşen Melda ÇOLAK
Assist. Prof. Dr. Kerem MERTOĞLU

İKSAD
Publishing House

PLANT MICROBE INTERACTIONS

EDITORS

Assoc. Prof. Dr. Ayşen Melda ÇOLAK
Assist. Prof. Dr. Kerem MERTOĞLU

AUTHORS

Prof. Dr. Halil İbrahim OĞUZ
Assoc. Prof. Dr. Asuman ARSLAN DURU
Assoc. Prof. Dr. Harun ÖZER
Assist. Prof. Dr. Aykut ŞENER
Assist. Prof. Dr. Barış KAKİ
Assist. Prof. Dr. Betül AYDIN
Assist. Prof. Dr. Burcu Begüm KENANOĞLU
Assist. Prof. Dr. Derya ÖĞÜT YAVUZ
Assist. Prof. Dr. Nurdoğan TOPAL
Assist. Prof. Dr. Hasret GÜNEŞ
Assist. Prof. Dr. Havva DİNLER
Dr. Alper DURMAZ
Dr. Ayşe UYSAL MORCA
Dr. Yunus KORKOM
MSc Medical Biochemistry Emine İncilay TORUNOĞLU
MSc Agricultural Engineer Şerife Nergis BAYAR
Res. Assist. Erdi Can AYTAR
Graduate Student Abdurrahman AY
Graduate Student Aslıhan Çilingir TÜTÜNCÜ
Graduate Student Bahadır YILMAZ
Graduate Student Sedanur YILMAZ

İKSAD
Publishing House

Institution of Economic Development and Social Researches Publications®
(The Licence Number of Publicator: 2014/31220)
TÜRKİYE TR: +90 342 606 06 75
USA: +1 631 685 0 853
E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

ISBN: 978-625-367-581-3
Cover Design: Arzu ALTUNTAŞ
December / 2023
Ankara / Türkiye
Size = 16x24 cm

CONTENTS

PREFACE

Contrary to the continuous increase in the world population, there is a constant decrease in arable areas. This situation has led to the necessity of using existing agricultural yards in the most effective way. As a matter of fact, when the production figures are examined, an increase in crop production is observed despite the decrease in agricultural areas. This is only possible by increasing productivity per unit area. The most important factor in increasing productivity was the introduction of new developed varieties into agricultural production. However, in parallel, farmers have also developed cultural practices that lead to increased productivity in agricultural production. However, recent deteriorations in natural resources and ecosystem balance have led to questioning the reliability of current practices. Dangerous situations such as eutrophication of groundwater due to excessive fertilization, residue problems caused by excessive use of pesticide active ingredients, and the development of new breeds of resistant disease/pest agents endanger sustainability. However, if we act consciously, there will be no need to experience these negative situations, and it will be possible to reduce production costs. How Does? Of course, with the use of microorganisms that make positive contributions by establishing a positive relationship with the plants. Some microorganisms have positive effects on the rhizosphere environment in the plant root zone, and some have positive effects on the phyllosphere in the above-ground parts, providing positive effects on nutrition and resistance. Even though certain microbes grow inside plant roots, their extensions spread to other plants in the surrounding soil. Plants share and communicate through this branching. Furthermore, these extensions that grow deeply contribute significantly to plant nutrition and drought resistance. In order to prevent the usage of pesticides, some of the advantageous microorganisms play a crucial role in decreasing disease or pest populations as they are natural enemies of other microbes that cause disease.In this book, important information is given about some microorganisms that can establish positive relationships with plants in different ways and show different positive effects. We express our respect on behalf of all writers and hope that the book we are the editors of will benefit the entire agricultural community. We also declare that we are open to any kind of feedback regarding the book.

EDITORS
Associate Prof. Dr. Ayşen Melda ÇOLAK
Assistant Prof. Dr. Kerem MERTOĞLU

CHAPTER 1

PLANT DISEASES OF FRUIT TREES: USE OF BENEFICIAL MICROORGANISMS FOR PRE- AND POSTHARVEST BIOLOGICAL CONTROL

Hasret GÜNEŞ[1]
Halil İbrahim OĞUZ[2]

DOI: https://dx.doi.org/10.5281/zenodo.10450459

---

[1] Adıyaman University, Faculty of Agriculture, Department of Plant Protection, Adıyaman, Türkiye hasretgunes@adiyaman.edu.tr Orcid ID: 0000-0003-3155-2695
[2] Adıyaman University, Faculty of Agriculture, Department of Horticulture, Adıyaman, Türkiye hioguz@adiyaman.edu.tr Orcid ID: 0000-0003-2213-7449

INTRODUCTION

Recent severe changes in climate pose a serious risk to the quantity and quality of agricultural production and jeopardize global food security. Plants are also more susceptible to disease attacks due to abiotic stress factors brought about by climate change (Ertürk, 2022). Increasing temperatures due to climate change are the agricultural production sector that affects fruit-growing agriculture the most. The increase in temperatures and changes in precipitation regime have led to an increase in disease agents in fruits. Due to this increase, the search for new methods in the fight against diseases in fruit growing has started, and there is an increase in R&D studies on environmentally friendly control methods. Fruit and vegetable production suffers significant losses due to agricultural diseases during plant cultivation, product processing, product transportation, and storage. However, synthetic fungicides are applied unconsciously and frequently to control diseases in these agricultural products. Although these fungicides are successful in the treatment of phytopathogen-borne diseases, they cause environmental pollution, create disease resistance, disrupt the ecological balance, leave harmful residues in soil, water, and atmosphere, and are economically costly and unsustainable alternatives (Sharma et al., 2009; Villarreal-Delgado et al., 2018). Therefore, it is highly desirable to generate innovative, environmentally friendly, sustainable solutions that focus on eliminating or reducing the use of synthetic fungicides in agriculture (Santoyo et al., 2012).

Biofertilizers are divided into different groups according to their functions and the nutrients they provide to plants. The contribution of biofertilizers to plant development and the rhizosphere is quite high (Al-Hchami and Salloom, 2023). Beneficial microorganisms living in the rhizosphere promote plant growth and increase resistance to many plant diseases. Among these microorganisms, Plant Growth Promoting Rhizobacteria increases crop productivity and reduces the use of chemical fertilizers and herbicides (Kloepper et al. 1980; Ashrafuzzaman et al., 2009).

There are many reasons for yield losses in fruit trees. When we list them, biotic and abiotic stresses come first. Biotic factors cause significant economic losses in both fruit yield and quality. Root, stem, leaf, and fruit health are the most important factors in pre-harvest yield losses. The use of environmentally friendly, sustainable biological practices in the control of diseases in fruit trees (root, stem, leaf, branch, fruit) has been increasing in recent years.

Pre-and post-harvest fruit loss due to phytopathogenic fungi is now thought to account for more than half of all agricultural fruit production (Zhang et al., 2017). High rates of microbial contamination are the primary cause of low fruit yield and quality. Mycotoxin-producing fungal diseases have a detrimental impact on both the economy and public health. Mycotoxins are dangerous chemicals that can pathologically damage human health. They are produced when fungi undergo secondary metabolism (Ismaiel and Papenbrock, 2015; Dwiastuti et al., 2021). Pre- and post-harvest fruit loss due to phytopathogenic fungi is now thought to account for more than half of all agricultural fruit production (Zhang et al., 2017).

1. Pre-Harvest Diseases in Fruit Trees

Rosellinia necatrix Bearl ex Prill is an important soil-borne root pathogen affecting many commercial fruit tree species such as apple, cherry, almond, peach, plum, pomegranate, grape, pear, olive, avocado, and grapevine. This fungus, the cause of white root rot disease, causes wilting and eventual death of host plants (Kanda et al., 2003; Ten Hoopen and Krauss, 2006; Cruz et al., 2014).

Plant diseases that damage fruit crops, including citrus, apples, strawberries, and grapes, can appear in the field or the days following harvest (Miskiyah and Broto, 2010; Dwiastuti et al., 2021). Studies on citrus have shown that Citrus Tristeza Virus infections like sticky pathogenic microbes such as *Rhizoctonia* sp., *Alternaria* sp., *Phytophthora* sp., *Pythium* sp., and *Xylella* sp. cause seeds to rot and sometimes even fail to germinate; those that grow develop poorly and eventually wither (Ahmed et al., 2012; de Sousa and Granada, 2023).

Various fruit trees are grown in Adıyaman. Various phytopathogenic plant diseases are encountered, especially pre-harvest and post-harvest. Anthracnose (*Colletotrichum gloeosporioides*), Leaf rust (*Tranzschelia discolor* f. sp. *dulcis*), powdery mildew (*Sphaerotheca pannosa*), flesh spot disease (*Polystigma ochraceum*), monilia (*Monilinia Laxa*), and almond branch cancer (*Pseudomonas amygdali*) are among the common diseases that affect almond trees (Gramaje et al., 2012; Karaat et al., 2021). Figure 1 shows important diseases of almonds.

Figure 1. a) Anthracnose (*Colletotrichum gloeosporioides*) b) Leaf rust (*Tranzschelia discolor* f. sp. *dulcis*), c) *Armillaria*, d) *Phytophthora*, e) *Alternaria* leaf spot, f) *Vertiticellium* (Ahmad et al., 2022).

The following are common diseases that affect walnut trees: leaf spot (*Microstroma juglandis*), bacterial blight (*Xanthomonas arboricola* pv. *juglandis*), anthracnose (*Gnomonia leptospira*), root rot (*Phytophthora* spp.), *Armillaria* root rot (*Armillaria mellea*), leaf spot (*Microstroma juglandis*). Important diseases seen in walnut in Figure 2.

Figure 2. Important diseases seen in walnut (Kałużna et al., 2021)

Olive trees are susceptible to several common diseases, including *Pseudomonas savastanoi* pv. *savastanoi*, anthracnose (*Gloeosporium olivarum* Alm.), and *Verticillium* wilt (*Verticillium dahliae*). The three most prevalent diseases affecting grapevines are retrograde death (*Eutypa lata*), gangrene disease (*Dothiorella* spp.), and vine disease (*Phaeomoniella chlamydospora*) (Camps et al., 2010; Wunderlich et al., 2011).

2. Post-harvest Diseases in Fruit Trees

Inadequate post-harvest fruit handling can cause rotting or other physical damage from falls during transportation. Such physiological damage facilitates the spread of the fungi. Many fruits are contaminated with pathogens during storage due to improper packaging, bumping, early harvesting, and chemical changes (Miskiyah and Broto, 2010). Especially, in less developed countries - especially in humid climates - post-harvest food losses account for around 30% of production (Bradford et al., 2018).

They cause plant death in immature trees and loss of quality and yield in established trees. Farmers are advised to use chemical pesticides such as Captan, Apon, and Dazomet to combat the causes of diseases in citrus (Gade et al., 2012). Biocontrol microbes in fruit trees play a critical role in reducing the incidence of postharvest diseases and pre-harvest phytopathogenic microbial infections. Due to their high nutrient and water content, postharvest fruit trees are more vulnerable to microbial pathogen infection after harvest (Talibi et al., 2014). Postharvest fruit rot in citrus orchards can be caused by diseases such as *Penicillium* spp. (Wang et al., 2022), *A. alternata, P. citrophthora*, and *C. gloeosporioides* (Shinde and Sadgir, 2016; Ferreira et al., 2020). Citrus has been associated with about 20 postharvest diseases, which cause enormous financial losses of about 20-25% (Devi and Kumari, 2015; Bazioli et al., 2019). Important diseases of pears are black spot (*Venturia pyrina*), root rot (*A. mellea*), and mammalian rust (*Gymnosporangium fuscum*). *Leucostoma cincta* [*Cytospora cincta* (anamorph)] causing cancer and backward death in apples, root rot (*Armillaria mellea*), apple blackleg disease caused by *V. inaequalis* in apples depends on the severity of infection in leaves, fruits, and shoots. Important diseases seen in apples after harvest are given in Figure 3.

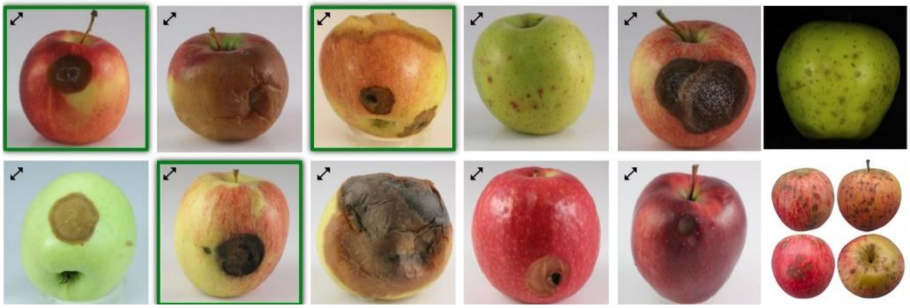


Figure 3. Important diseases seen in apples after harvest (Buyukarikan and Ulker, 2022; Argenta et al., 2023).

The question for plant pathologists is to prehend this moment to both bring out and help biological control procedures as a feasible alternative to fungicides for the control of postharvest diseases of fruits and vegetables. Achieve this, a concerted research and development effort will be needed at various government, university, and industry laboratories, which will required a substantial increase in funding and cooperation. Host opportunities are open for the development of biological control procedures for post-harvest diseases of fruits and vegetables as an alternative to fungicides. investigation of natural plant products as fungicides should be investigated. Also, a basic understanding of resistance and defense strategies in harvested commodities should expose plurality of new approaches for control of postharvest diseases.

3. Beneficial Microorganisms in Fruit Trees

Soil biology, with its dynamic and complex structure, varies according to conditions and increases soil fertility by interacting with living elements. Microorganisms have a high density in the rhizosphere region, which we call the plant root zone in the soil. Beneficial microorganisms, which are also considered plant biostimulants depending on the type of microbes, can reduce the effect of biotic-abiotic stress factors and support plant growth independently of nutrients in the soil (Ertürk, 2022). Arbuscular mycorrhizal fungi (AMF), *Trichoderma*, and plant growth promoting *Rhizobacteria* are important biostimulants in this group (Jacoby et al., 2017). They are primarily used to feed plants or to protect plant-tree fruits against diseases (Sible et al., 2021; Bartucca et al., 2022; Güneş, 2023).

In addition to fixing nitrogen in the atmosphere and using siderophores to absorb iron, beneficial microbes also suppress soil pathogens, increase plant nutrients and water availability, help break down organic waste, form antibiotic substances, and reduce the uptake of heavy metals. Biofertilizers are useful living microorganisms that improve soil fertility, promote plant growth, and decrease biotic and abiotic stress factors in various plants, including fruit trees. They are also environmentally friendly, sustainable techniques (Ertürk, 2022; Gunes et al., 2023).

Biological control is a very important strategy in integrated disease management. This is because it is an excellent alternative to chemical control and a sustainable practice. Research clearly shows the effectiveness of Arbuscular Mycorrhizal Fungi (AMF) in plant disease management, either as a single application or as an AMF-beneficial microorganism association. AMF can establish symbiotic relationships with 80% of terrestrial plants, have a mutualistic relationship with plant roots, and have successful and sustainable applications in many biotic and abiotic stress factors (St-Arnaud et al., 1994; Azcón-Aguilar and Barea, 1997). The beneficial microbe *Trichoderma* is one of the most widely used plant pathogen antagonists in various biological applications. It has been demonstrated to increase nutrient uptake, promote root growth, and enhance plant growth. Through a variety of biological mechanisms, such as the inactivation of pathogen enzymes, competition for nutritional space, mycoparasitism, and synthesis of inhibitory compounds, *Trichoderma* species help confer resistance to plant infections (Roco and Pérez, 2001; Yedidia et al., 2000). On the other hand, Plant Growth Promoting Rhizobacteria (PGPR) have a direct impact on plants because they aid in nutrient uptake, effectively combat plant diseases, and encourage nitrogen fixation (Alagawadi and Gaur, 1992; Bashan and de-Bashan, 2005). Therefore, environmentally friendly biocontrol agents applied pre-harvest are a good tactic for fruit trees that may be adversely affected during post-harvest transportation or storage.

This book chapter evaluates the results of studies to demonstrate the efficacy of beneficial microbes such as Arbuscular Mycorrhizal Fungi (AMF), *Trichoderma* species, and Plant Growth Promoting Rhizobacteria (PGPR) on fruit cultivation and postharvest plant diseases.

4. Fruit Culture and Fruit Tree Diseases: The Role of Arbuscular Mycorrhizal Fungi (AMF)

In fruit trees, some microbial species function as phytopathogens, and some microbial interactions promote the growth of organic matter (Pandit et al., 2022). When used as biocontrols in fruit trees, beneficial non-pathogenic microbes can lessen the effects of numerous stressors (Ruano-Rosa and Mercado-Blanco, 2015). You can apply these advantageous microbes to soil, seeds, fruits, and leaves. The detrimental effects of plant disease are decreased by these microbes, which also emit biological substances, inhibit the growth of fruit tree diseases, and create systemic resistance to them (Schmidt et al., 2019; de Sousa and Granada, 2023).

AMF, one of the beneficial microbes used in sustainable agricultural methods, not only develops a mutualistic relationship with plants but also makes a significant growth contribution to them. Thanks to the hyphae that AMF forms in symbiotic harmony with plant roots, it expands the surface area of plant roots and promotes mineral uptake from the soil, allowing plants to grow more (Ertürk, 2022). According to Türkmen et al. (2008), AMF is also an important microbe in terms of sustainability as it makes plants more resistant to environmental pollutants such as plant diseases, negative pH, heavy metals, and salt stress. According to Rouphael et al. (2015), they also secrete phosphatase and other organic molecules to increase the P concentration in the soil.

Pythium ultimum Trow, *Rhizoctonia solani* Khun and *Phytophthora* spp. are just a few of the plant diseases where AMF is crucial for their suppression (Cordier et al., 1996; Trotta et al., 1996; Yao et al., 2002; Cruz et al., 2014). *J.lividum*, some bacteria of the genus Bacillus and *Gigaspora margarita* AMF spores play an important role in phosphorus (P) uptake and disease suppression (antagonism), for example, *Paenibacillus* sp. and *Glomus mosseae* AMF have been found to have an antagonistic effect on soilborne pathogens in sorghum (Budi et al., 1999; Cruz et al., 2008). Three major fungal diseases that were identified during the pre-harvest seedling phase in strawberries, namely *Fusarium oxysporum, Alternaria alternata*, and *Rhizoctonia fragariae*, were evaluated together with the efficacy of *Gigaspora margarita* and *Funneliformis mosseae* AMF species against them. According to research, AMF improved the wet and dry weight of plants as well as the phosphorus value (P) and total phenol antioxidant value (Demir et al., 2023).

Research has demonstrated that AMF colonization enables strawberry (Fragaria × ananassa) plants to flower and fruit earlier, produce more sugar, anthocyanin, and photosynthesis, and improve plant development (Hršelová et al., 1990; Borkowska, 2002; Sohn et al., 2003; Castellanos-Morales et al., 2010).

5. The Function of the Beneficial Microbe *Trichoderma* in Fruit Tree Diseases

Biological control agents are a crucial part of sustainable agricultural operations for farmers. One of these microscopic entities is called *Trichoderma*, and it works via a variety of processes, including competition, mycoparasitism, antibiosis, inducing host resistance, and enzyme inhibition (Niu et al. 2020; Kang et al. 2021; Ben M'henni et al., 2022). Among the most well-known helpful fungi is *Trichoderma*, which has a large number of species that inhabit plant tissue and the rhizosphere (Meraj-ul and Nandkar 2012). This fungi produces secondary metabolites. While it shows mechanism activity against *Phytophthora* species through competition, antibiosis and parasitism, it has been clearly established that it has antagonistic effect against *Pythium* species (Suarez et al. 2005; Mpika et al. 2009). Use the plant-*Trichoderma* symbiont promotes plant growth and increases stress tolerance (Ramírez-Valdespino et al., 2019).

According to Aslan et al. (2021), different *Trichoderma* species (*Trichoderma harzianum*, *Trichoderma viride*) were effective against *Armillaria* root rot and *Desarmillaria*. *Trichoderma* is a useful agent for controlling *Fusarium* root rot disease caused by *F.solani* in olive trees.

6. Fruit Tree Diseases with Rhizobacteria-Promoting Plant Growth (PGPR)

Many years have been dedicated to researching the link between bacteria and plants. Plants can choose which microbes to colonize beneficial bacteria by living in plant tissues, a process known as endophytes. Bacteria have been shown to have numerous positive effects on plant growth and health (De Souza et al., 2016; Santoyo et al., 2016). According to Hardoim et al. (2015), endophytic behavior is functionally defined as any bacterium that can be isolated from plant tissue or removed from its disinfected surface is an endophyte as long as it does not visibly harm the plant.

Plant Growth Promoting Rhizobacteria (PGPR) comprises several functional and taxonomic groups (Ghosh et al., 2003), the most widely used and researched being *Pseudomonas fluorescens* (Duijff et al., 1997; Vazquez et al., 2000). In soil, nutrient uptake directly promotes plant growth through many pathways such as nitrogen fixation, phytohormone production, and IAA (Glick, 1995), while phytopathogens act indirectly (Benizri et al., 2001).

Few studies (Lingua et al., 2013; Bona et al., 2015) have examined the effect of PGPR on strawberries. A synergistic effect on strawberry growth after co-inoculation with AMF and *Pseudomonas putida* and promotion of AMF root colonization by *Agrobacterium radiobacter* has been previously reported (Vosatka et al., 2000).

In another postharvest study in strawberries (Fragaria × ananassa var.), the effects of AMF (*Funneliformis mosseae*, *Septoglomus viscosum*, and *Rhizophagus irregularis*) and three different *Pseudomonas* sp. strains (19Fv1t, 5Vm1K and Pf4) on plant growth parameters, fruit production and quality were investigated. As a result of the study, plant growth, fruit nutritional quality, sugar, and anthocyanin concentrations increased, and *Rhizophagus irregularis* established more symbiotic relationships with plant roots than other species in terms of root colonization (Todeschini et al., 2018).

CONCLUSION

Before starting research on fruit tree diseases caused by microbes, care should be taken to choose the best plant-microorganism combination, cut back on chemical inputs, come up with concepts to improve soil sustainability from an ecological and financial standpoint, increase fruit quality and productivity, and think about disease control. Therefore this book chapter presents common pre-harvest diseases of fruit trees, plant biostimulants in the rhizosphere, their effects, benefits, and recommendations for reducing pesticide use. At the same time, the benefits of plant nutrients of plant biostimulants to fruit trees are indicated. The effect of using biocontrol methods to combat diseases in plant soil parts in terms of environmental and plant health is indicated. In addition, it was determined that the use of biostimulants especially in storage processes in the fight against storage-borne disease agents that negatively affect post-harvest physiology has significant beneficial effects in terms of fruit quality and storage time.

REFERENCES

Ahmad, S., Muhammad, E., Ahmad, I., Haq, M. E., Rehman, M.A., Haq, I.U. and Zeeshan, M.A. (2022). Current status of fungal dıseases of almond ın dıstrıct loralaı and zıarat. *Pakistan Journal of Phytopathology*, 34(2): 349-360.

Ahmed, Y., D'ONGHIA, A.M., Ippolito, A., El Shimy, H., Cirvilleri, G. and Yaseen, T. (2012). *Phytophthora nicotianae* is the predominant *Phytophthora* species in citrus nurseries in Egypt. *Phytopathologia Mediterranea*, 519-527.

Alagawadi, A.R. and Gaur, A.C. (1992). Inoculation of *Azospirillum brasilense* and phosphate-solubilizing bacteria on yield of sorghum [*Sorghum bicolor* (L.) Moench] in dry land. Tropical Agriculture.

Al-Hchami, S.H. and Salloom, Y.F. (2023). Biofertilizers, types, Benefits, and their role in improving the growth of fruit trees and vegetative plants. *Kirkuk University Journal for Agricultural Sciences*, 14(3): 47-56.

Argenta, L.C., Wood, R.M., Mattheis, J.P., Thewes, F.R., Nesi, C.N. and Neuwald, D. A. (2023). Factors affecting development of disorders expressed after storage of ‘Gala’apple fruit. *Postharvest Biology and Technology*, 204: 112439.

Ashrafuzzaman, M., Hossen, F.A., Ismail, M.R., Hoque, A., Islam, M.Z., Shahidullah, S. M. and Meon, S. (2009). Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology*, 8(7).

Azcón-Aguilar, C. and Barea, J.M. (1997). Applying mycorrhiza biotechnology to horticulture: significance and potentials. *Scientia Horticulturae*, 68(1-4): 1-24.

Bartucca, M.L., Mimmo, T., Cesco, S. and Del Buono, D. (2016). Nitrate removal from polluted water by using a vegetated floating system. *Science of the Total Environment*, 542: 803-808

Bashan, Y. and De-Bashan, L.E. (2005). Plant growth-promoting. *Encyclopedia of soils in the environment*, 1: 103-115.

Ben M'henni, Y., Salem, I.B., Souli, M., Tounsi, S., Debieu, D., Fillinger, S. and Boughalleb-M'hamdi, N. (2022). Biocontrol and Growth Promotion Potential of Combined Application of Trichoderma simmonsii and Aspergillus westerdijkiae Against Apple Tree Dieback Disease. *PhytoFrontiers*, 2(3): 268-279.

Benizri, E., Baudoin, E. and Guckert, A. (2001). Root colonization by inoculated plant growth promoting rhizobacteria. *Biocontrol Sci. Technol*. 11: 557–574.

Bona, E., Lingua, G., Manassero, P., Cantamessa, S., Marsano, F. and Todeschini, V. (2015). AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. *Mycorrhiza* 25: 181–193.

Borkowska, B. (2002). Growth and photosynthetic activity of micropropagated strawberry plants inoculated with endomycorrhizal fungi (AMF) and growing under drought stress. *Acta physiologiae plantarum*, 24: 365-370.

Bradford, K.J., Dahal, P., Van Asbrouck, J., Kunusoth, K., Bello, P., Thompson, J. and Wu, F. (2020). The dry chain: Reducing postharvest losses and improving food safety in humid climates. *In Food industry wastes*, 375-389. Academic Press.

Budi, S. V., Van Tuinen, D., Martinotti, G. and Gianinazzi, S. (1999). Isolation from the Sorghum bicolor mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soilborne fungal pathogens. *Applied and environmental microbiology*, 65(11): 5148-5150.

Buyukarikan, B. and Ulker, E. (2022). Classification of physiological disorders in apples fruit using a hybrid model based on convolutional neural network and machine learning methods. *Neural Computing and Applications*, 34(19): 16973-16988.

Camps, C., Kappel, C., Lecomte, P., Léon, C., Gomès, E., Coutos-Thévenot, P. and Delrot, S. (2010). A transcriptomic study of grapevine (*Vitis vinifera* cv. Cabernet-Sauvignon) interaction with the vascular ascomycete fungi Eutypa lata. *Journal of experimental botany*, 61(6): 1719-1737.

Castellanos-Morales, V., Villegas, J., Wendelin, S., Vierheilig, H., Eder, R. and Cárdenas-Navarro, R. (2010). Root colonisation by the arbuscular mycorrhizal fungus Glomus intraradices alters the quality of strawberry fruits (Fragaria× ananassa Duch.) at different nitrogen levels. *Journal of the Science of Food and Agriculture*, 90(11): 1774-1782.

Cordier, C., Gianinazzi, S. and Gianinazzi-Pearson, V. (1996). Colonisation patterns of root tissues by *Phytophthora nicotianae* var. parasitica related to reduced disease in mycorrhizal tomato. *Plant and soil*, 185: 223-232.

Cruz, A.F., Horii, S., Ochiai, S., Yasuda, A. and Ishii, T. (2008). Isolation and analysis of bacteria associated with spores of *Gigaspora margarita*. *Journal of applied microbiology*, 104(6): 1711-1717.

Cruz, A.F., Soares, W.R.O. and Blum, L.E.B. (2014). Impact of the arbuscular mycorrhizal fungi and bacteria on biocontrol of white root rot in fruit seedlings. *J Plant Physiol Pathol* 2: 1- 2.

Demir, S., Durak, E. D., Güneş, H., Boyno, G., Mulet, J.M., Rezaee Danesh, Y. and Porcel, R. (2023). Biological control of three fungal diseases in strawberry (Fragaria× ananassa) with arbuscular mycorrhizal fungi. *Agronomy,* 13(9): 2439.

de Souza, R.S.C., Okura, V.K., Armanhi, J.S.L., Jorrín, B., Lozano, N., Da Silva, M.J., González-Guerrero, M., deAraújo, L.M., Verza, N.J., Bagheri, H.C., Imperial, J. and Arruda, P. (2016). Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. *Scientific Reports*, 6(1): 28774.

de Sousa, M.A. and Granada, C.E. (2023). Biological control of pre-and post-harvest microbial diseases in Citrus by using beneficial microorganisms. *Biocontrol*, 68(1): 75-86.

Duijff, B.J., Gianinazzi-Pearson, V. and Lemanceau, P. (1997). Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol Pseudomonas fluorescens strain WCS417r. *New Phytol*. 135: 325–334.

Dwiastuti, M.E., Soesanto, L., Aji, T.G., Devy, N.F. and Hardiyanto. (2021). Biological control strategy for postharvest diseases of citrus, apples, grapes and strawberries fruits and application in Indonesia. *Egyptian Journal of Biological Pest Control*, 31: 1-12.

Ertürk, Y. (2022). Biological Fertilizers-Containing Beneficial Microorganisms in Fruit Culture. *Kırşehir Ahi Evran Üniversitesi Ziraat Fakültesi Dergisi*, 2(1): 71-92.

Ferreira, F.V., Herrmann-Andrade, A. M., Calabrese, C.D., Bello, F., Vázquez, D. and Musumeci, M. A. (2020). Effectiveness of Trichoderma strains isolated from the rhizosphere of citrus tree to control Alternaria alternata, *Colletotrichum gloeosporioides* and *Penicillium digitatum* A21 resistant to pyrimethanil in post-harvest oranges (Citrus sinensis L. (Osbeck)). *Journal of applied microbiology*, 129(3): 712-727.

Gade, R. M. (2012). Biological and chemical management of Phytophthora root rot/collar rot in citrus nursery. *The bioscan*, 7(4): 631-635.

Ghosh, S., Penterman, J.N., Little, R.D., Chavez, R. and Glick, B.R. (2003). Three newly isolated plant growth-promoting bacilli facilitate the seedling growth of canola, Brassica campestris. *Plant Physiol. Biochem*., 41: 277–281

Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.,* 41, 109–117.

Gramaje, D., Agustí-Brisach, C., Pérez-Sierra, A., Moralejo, E., Olmo, D., Mostert, L.I.Z.E.L., Damm, U. And Armengol, J. (2012). Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). *Persoonia-Molecular Phylogeny and Evolution of Fungi*, 28(1): 1-13.

Gunes, H., Demir, S., Erdinc, C. and Furan, M. A. (2023). Effects of Arbuscular Mycorrhızal Fungı (AMF) and Bıochar On the Growth of Pepper (*Capsicum annum* L.) Under Salt Stress. *Gesunde Pflanzen*, 1-13.

Güneş, H. (2023). Enhancing Phytoremediation Effect with Sustainable Biostimulants. A. Çelik, Z. Aslam, K. Bellitürk, A. Ahmad, Z.Mustaq (Ed.), Latest Studıes and Developments in Agrıculture and Envıronment (129-160). Iksad Press.

Hardoim, P.R., Van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., Döring, M. and Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and molecular biology reviews*, 79(3): 293-320.

Hršelová, H., Gryndler, M. and Vančura, V. (1990). Influence of inoculation with VA mycorrhizal fungus Glomus sp. on growth of strawberries and runner formation. *Agriculture, Ecosystems & Environment*, 29(1-4): 193-197.

Ismaiel, A. A. and Papenbrock, J. (2015). Mycotoxins: producing fungi and mechanisms of phytotoxicity. *Agriculture*, 5(3): 492-537.

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A. and Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. *Frontiers in plant science*, 8: 1617.

Kałużna, M., Fischer-Le Saux, M., Pothier, J.F., Jacques, M. A., Obradović, A., Tavares, F., & Stefani, E. (2021). Xanthomonas arboricola pv. juglandis and pv. corylina: Brothers or distant relatives? Genetic clues, epidemiology, and insights for disease management. Molecular Plant Pathology, 22(12), 1481-1499.

Kanda, S., Ishiguro, M., Kano, S., Aimi, T., Kitamoto, Y. and Morinaga, T. (2003). Heterothallic life cycle in the white root rot fungus Rosellinia necatrix. *Mycoscience*, 44: 389-395.

Kang, S., Lumactud, R., Li, N., Bell, T.H., Kim, H.S., Park, S.Y. and Lee, Y. H. (2021). Harnessing chemical ecology for environment-friendly crop protection. *Phytopathology*, 111(10): 1697-1710.

Karaat, Ş., Mehmet, Atay. and Tohumcu, E. (2021). Determination of Fungal Diseases Seen in Almond Production Areas in Adıyaman Province. *ADYUTAYAM Journal*, 9(1): 36-46.

Khan, M.A., Ali, M., Shah, M., Mahmood, T., Ahmad, M., Jhanjhi, N.Z., Bhuiyan, M.A.S. and Jaha, E. S. (2021). Machine learning-based detection and classification of walnut fungi diseases. *Intelligent Automation & Soft Computing*, 30(3): 771-785.

Khasanov, B.A., Safarov, A. A. and Boyjigitov, F. M. (2019). Fungal diseases of persian walnut in Uzbekistan and their control. *Indian Journal of Pure & Applied Biosciences*, 7(5): 327-334.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, 286(5776): 885-886.

Lingua, G., Bona, E., Manassero, P., Marsano, F., Todeschini, V., Cantamessa, S., Copetta, A., D'Agostino, G.,Gamalero, E. and Berta, G. (2013). Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads increases anthocyanin concentration in strawberry fruits (Fragaria x ananassa var. Selva) in conditions of reduced fertilization. *International Journal of Molecular Sciences,* 14: 16207–16225.

Niu, B., Wang, W., Yuan, Z., Sederoff, R.R., Sederoff, H., Chiang, V.L. and Borriss, R. (2020). Microbial interactions within multiple-strain biological control agents impact soil-borne plant disease. *Frontiers in Microbiology*, 11: 585404.

Pandit, M.A., Kumar, J., Gulati, S., Bhandari, N., Mehta, P., Katyal, R., Dogra Rawat, C., Mishra, V. and Kaur, J. (2022). Major biological control strategies for plant pathogens. *Pathogens,* 11(2): 273.

Poyraz, D. and Uysal-Morca, A. (2022). Research on the Control of Petri Disease in Vineyards. *The Journal of Turkish Phytopathology*, 50(2-3): 23-33.

Ramírez-Valdespino, C.A., Casas-Flores, S. and Olmedo-Monfil, V. (2019). *Trichoderma* as a model to study effector-like molecules. *Frontiers in microbiology*, 10: 1030.

Roco, A. and Pérez, L.M. (2001). In vitro biocontrol activity of Trichoderma harzianum on Alternaria alternata in the presence of growth regulators. *Electronic Journal of Biotechnology*, 4(2): 1-2.

Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., de Pascale, S., Bonini, P. and Colla, G. (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Scientia Horticulturae*, 196: 91-108.

Ruano-Rosa, D. and Mercado-Blanco, J. (2015). Combining biocontrol agents and organics amendments to manage soil-borne phytopathogens. Organic amendments and soil suppressiveness in plant disease management, 457-478.

Santoyo, G., Orozco-Mosqueda, M.D.C. and Govindappa, M. (2012). Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of Bacillus and Pseudomonas: a review. *Biocontrol Science and Technology*, 22(8): 855-872.

Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M. and Glick, B. R. (2016). Plant growth-promoting bacterial endophytes. *Microbiological research*, 183: 92-99.

Schmidt, T.M., Thomé, A.H.E., Sperotto, R.A. and Granada, C.E. (2019). Effect of rhizobia inoculation on the development of soil-borne pathogens infecting common bean plants. *European Journal of Plant Pathology*, 153(3): 687-694.

Sible, C.N., Seebauer, J.R. and Below, F.E. (2021). Plant biostimulants: A categorical review, their implications for row crop production, and relation to soil health indicators. *Agronomy*, 11(7): 1297.

Sharma, R.R., Singh, D. and Singh, R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological control*, 50(3): 205-221.

Shinde, S. B. and Sadgir, M. D. (2016). Efficiency of Pseudomonas fluorescens and Bacillus subtilis against Phytophthora spp. in citrus. *International journal of plant protection*, 9(1): 15-20.

Sohn, B.K., Kim, K.Y., Chung, S.J., Kim, W.S., Park, S.M., Kang, J.G., Rim, Y.S., Cho, J.S., Kim, T.H. and Lee, J.H. (2003). Effect of the different timing of AMF inoculation on plant growth and flower quality of chrysanthemum. *Scientia Horticulturae*, 98(2): 173-183.

Suárez, M. B., Sanz, L., Chamorro, M.I., Rey, M., González, F.J., Llobell, A. and Monte, E. (2005). Proteomic analysis of secreted proteins from Trichoderma harzianum: identification of a fungal cell wall-induced aspartic protease. *Fungal Genetics and Biology*, 42(11): 924-934.

St-Arnaud, M., Hamel, C. and Fortin, J.A. (1994). Inhibition of Pythium ultimum in roots and growth substrate of mycorrhizal Tagetes patula colonized with Glomus intraradices. *Canadian Journal of Plant Pathology*, 16(3): 187-194.

Talibi, I., Boubaker, H., Boudyach, E.H. and Ait Ben Aoumar, A. (2014). Alternative methods for the control of postharvest citrus diseases. *Journal of applied microbiology*, 117(1): 1-17.

Ten Hoopen, G.M. and Krauss, U. (2006). Biology and control of Rosellinia bunodes, Rosellinia necatrix and Rosellinia pepo: a review. *Crop protection*, 25(2): 89-107.

Trotta, A., Varese, G.C., Gnavi, E., Fusconi, A., Sampo, S. and Berta, G. (1996). Interactions between the soilborne root pathogen Phytophthora nicotianae var. parasitica and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. *Plant and soil*, 185, 199-209.

Todeschini, V., AitLahmidi, N., Mazzucco, E., Marsano, F., Gosetti, F., Robotti, E., Bona, E., Massa, N., Bonneau, L., Marengo, E., Wipf, D., Berta, G. and Lingua, G. (2018). Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality, and volatilome. *Frontiers in plant science,* 9: 1611.

Vazquez, P., Holguin, G., Puente, M. E., Lopez-Cortes, A., and Bashan, Y. (2000). Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol. Fertil. *Soils* 30: 460–468.

Villarreal-Delgado, M.F., Villa-Rodríguez, E. D., Cira-Chávez, L.A., Estrada-Alvarado, M.I., Parra-Cota, F.I. and Santos-Villalobos, S.D.L. (2018). The genus Bacillus as a biological control agent and its implications in the agricultural biosecurity. *Revista mexicana de fitopatología*, 36(1): 95-130.

Vosatka, M., Gryndler, M., Jansa, J. and Vohnik, M. (2000). Post vitro mycorrhization and bacterization of micropropagated strawberry, potato and azalea. Acta Horticulturae, 530: 313–324.

Wang, Z., Zhong, T., Chen, X., Xiang, X., Du, M., Zalán, Z. and Kan, J. (2022). Multiple pre-harvest applications of antagonist Pseudomonas fluorescens ZX induce resistance against blue and green molds in postharvest citrus fruit. *Lebensmittel-Wissenschaft & Technologie*, 155: 112922.

Wunderlich, N., Ash, G., Steel, C., Raman, A. and Savocchia, S. (2011). Association of Botryosphaeriaceae grapevine trunk disease fungi with the reproductive structures of Vitis vinifera. *VITIS-Journal of Grapevine Research*, 50(2): 89-96.

Yao, M., Tweddell, R. and Desilets, H. (2002). Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by Rhizoctonia solani. *Mycorrhiza*, 12: 235-242.

Yedidia, I., Benhamou, N., Kapulnik, Y. and Chet, I. (2000). Induction and accumulation of PR proteins activityduring early stages of root colonizationby the mycoparasite *Trichoderma harzianum* strain T-203. *Plant Physiology and Biochemistry*, 38(11): 863-873.

Zhang, H., Mahunu, G. K., Castoria, R., Apaliya, M.T. and Yang, Q. (2017). Augmentation of biocontrol agents with physical methods against postharvest diseases of fruits and vegetables *Trends in Food Science and Technology, 69-36.*

CHAPTER 2

EFFECT OF PHYTOGENIC ADDITIVES ON SILAGE MICROBIOLOGY

Asuman ARSLAN DURU[1]

DOI: https://dx.doi.org/10.5281/zenodo.10450462

---

[1] Department of Animal Science, Faculty of Agriculture, Uşak University, Uşak, Turkiye asuman.duru@usak.edu.tr Orcid ID 0000-0002-7290-1719

INTRODUCTION

Silage is a roughage obtained by fermentation of plant products with high water content, agricultural and industrial residues by lactic acid bacteria in anaerobic environment. The age-old practice of ensiling fresh forage to preserve it has drawn more attention. Ensiling is essentially a fermentation process in which many different groups of microorganisms play an important role. Factors such as the chemical and microbiological structure of the forage to be ensiled are effective on the quality of silage.

There are three interrelated factors in ensiling period. These are forage material to be ensiled, ensiling manages and fermentation process. To obtain a quality silage, all these factors should be emphasized meticulously. In addition, the populations of epiphytic microorganisms contained in fresh plants are highly variable and are influenced by various factors such as plant species, ripening period, weather conditions, harvesting, wilting, and shredding (Spoelstra and Hindle, 1989).

Ensiling has proven to be a valid process for utilization of agricultural by-products due to its ability to preserve nutrients (Xue et al, 2020). Effective silage fermentation is a natural process that lowers pH as quickly as possible, prevents microorganisms that cause decay, and preserves the maximum amount of nutrients by using epiphyte lactic acid bacteria to ferment the water-soluble carbohydrates in the forage into a variety of products, primarily lactic acid (Merry and Davies, 1999). Water-soluble carbohydrates are broken down by microorganisms to create organic acids, which lower pH and stop the growth of putrefactive bacteria to generate silage (McDonald et al., 1991; Muck et al., 2018). This process yields silage. Because it may retain nutrients, ensiling has been shown to be a popular method for using agricultural leftovers (Lv et al., 2020; Xue et al., 2020).

Lactic acid bacteria are the most important microflora in silage during the fermentation process. Because the ensiled material is protected by lactic acid. Other microorganisms, especially members of the Enterobacteriaceae family, Clostridial spores, yeasts, and molds, have a negative effect on silage fermentation. These microorganisms compete with lactic acid bacteria to utilize fermentable carbohydrates and their end products to negatively affect silage fermentation (Weinberg and Muck, 1996).

Recently, a variety of microbial additives are also available to help preserve silages. It is also important to know their possible effects on silage fermentation, preservation, and their possible use in livestock nutrition.

Silage Microbiology

Silage micro flora plays a key role in achieving good quality fermentation in silages. In general, microorganism flora is divided into desirable and undesirable microorganisms. Desirable microorganisms in silage are mainly lactic acid bacteria. Lactic acid bacteria provide the preservation of silages with their metabolic products, lactic acids. Undesirable microorganisms are responsible for anaerobic decay (such as clostridia and enterobacteria) and aerobic decay (such as yeasts, molds, and listeria). These microorganisms causing silage decay not only to reduce the nutritional value of silage, but also significantly reduce animal health and the quantity and quality of the animal product (Driehuis and Oude Elferink, 2000).

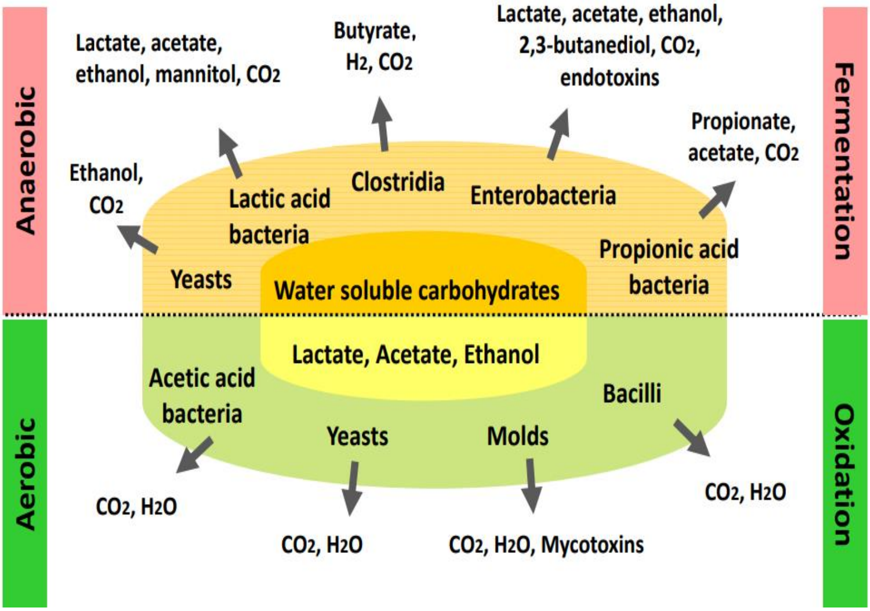


Figure 1. Silage microorganisms (Merry and Davies, 1999)

During the fermentation period, different types of bacteria they become dominant at different times, fermentation can only take place if sufficient water-soluble carbohydrates are provided.

Lactic Acid Bacteria

The epiphytic flora on plant material is the source of lactic acid bacteria, and soil is the primary source. Numerous factors, including the plant's dry matter content, growth stage, and the availability of nutrients on the leaves, as well as soil characteristics, altitude, and climatic conditions, affect the density of lactic acid bacteria on forage (Carr et al., 2002). Although the number of lactic bacteria is low in fresh material, this value can reach about 8-9 times in the advanced stages of fermentation and become the dominant microflora in silage. There may also be significant increases in the number of these microorganisms during harvesting and wilting of fresh material (Henderson et al., 1972).

Most species of lactic acid bacteria linked to silage are found in the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Enterococcus* (Pahlow et al., 2003). In addition, the genera *Lactococcus* and *Streptococcus* also contain some important lactic acid bacteria species.

They all ferment sugars, but other products, notably acetic acid, ethanol, and carbon dioxide, are common. They are usually grouped into homofermentative and heterofermentative according to the products of fermenting glucose. One mole of glucose is used by homofermentative ones to produce two moles of lactic acid. One mole of glucose can yield one mole of lactic acid, one mole of carbon dioxide, and one mole of either acetic acid or ethanol by heterofermentative bacteria (Muck, 2010).

The full potential of lactic acid bacteria as well as organic acid production is not yet known, but inhibition of mycotoxin production by fungi appears to be due to microbial competition, low pH, nutrient depletion, and the generation of heat-stable metabolites (Dogi et al., 2013). Since the water-soluble carbohydrate content of the forage to be ensiled is rapidly broken down by lactic acid bacteria during fermentation and converted into lactic acid, it is desired to have sufficient abundance. Otherwise, lactic acid bacteria cannot become the dominant microflora in silages. Lactic acid bacteria are a successful application as an inhibitor of undesirable microorganisms due to their essential role in silage.

Enterobacteria

Enterobacteria are normally effective in silages with a pH of around 6-7, while most of them are not effective when the pH is below 5. Therefore, a dense population of Enterobacteria is usually present in the plant during the first hours of ensiling (Lin et al., 1992). After the first few days of the fermentation period, the pH starts to decrease, and their numbers decrease rapidly, and they do not cause any problems (Filya, 2001).

Clostridia

Clostridia also have a significant effect on silage quality. Clostridia can be divided into two main groups: saccharolytic and proteolytic clostridia. Saccharolytic clostridia convert sugars and organic acids in the plant into butyric acid. Proteolytic clostridia ferment amino acids and volatile organic acids (Woolford, 1984).

Clostridia species microorganisms are the most important competitors of lactic acid bacteria during silage fermentation because they use the carbohydrates used by these microorganisms. However, because of catabolism of amino acids, they decrease the value of feed, cause energy loss and increase the pH of the environment. These microorganisms can develop in silage, but they can also be found in soil and feces, and they can infect the forage plant to be ensiled in these ways (Weinberg and Muck, 1996).

Listeria

Listeria bacteria have a negative effect on the feed value and hygienic quality of silage and pose a great danger to animal and human health. Therefore, these bacteria, like Clostridium bacteria, are never desirable in silage. Many species of Listeria have been identified in silage samples and it has been found that Listeria monocytogenes is the most common and this species causes inflammation in the brain tissue and paralysis in animals. Listeria monocytogenes is a non-spore-forming and rod-shaped bacterium and is commonly found in rotten fruits and vegetables, feces and soil (McDonald, 1981).

Yeasts

Yeasts are the most important aerobic microorganisms in the palnt when considering silage quality. Yeasts thrive on soluble substrates, of which sugars and lactic acid are the most crucial in silage When oxygen comes into contact with silage during feeding or storage, yeasts usually start to grow first. This is because many yeasts can grow at pH 3.5, well below the pH of most silage. Such acidic environments are conducive to the growth of mold and acetic acid bacteria, though the latter are uncommon and grow far more slowly than the former. The pH of the silage rises as yeast strains that can use lactic acid aerobically grow. This paves the way for the growth of other spoilage (aerobic) microorganisms, especially when the pH rises above 4.5 (Muck, 2010).

Counting yeasts in silages can be useful because, high yeast number in silages are usually linked to high ethanol concentrations and their numbers are typically inversely correlated with the aerobic stability of silages. Also, high yeast content is linked to subpar animal performance (Kung et al, 2018).

Acetic acid bacteria

Another group of microorganisms that are effective on aerobic spoilage are acetic acid bacteria. Acetic acid bacteria are acid-tolerant, aerobic bacteria. It is reported that these bacteria have inhibitory effects on yeast growth and yeast growth is not observed when Acetobacter species are present in the silage (Spoelstra et al., 1988).

Bacilli

Bacilli are facultative anaerobic or endospore-forming aerobic bacteria. *B. lentus, B. licheniformis, Bacillus cereus, B. sphaericus, B. polymyxa,* and *B firmus* are typically associated with silage (Woolford et al., 1979). Some species can also grow under anaerobic conditions. Bacillus spp., which are more tolerant to heat than yeasts, can replace yeasts with an increase in temperature during silage degradation (Basmacıglu and Ergul, 2002).

Mold

Molds are filamentous and aerobic fungi found on the product. Molds should be treated with extreme caution as they produce mycotoxins in silage.

Before ensiling, forage can harbor a variety of fungi that can infect plants growing in the field and produce mycotoxins. Additional fungal contamination can occur during harvesting and chopping from soil, ambient dust and air. These fungi and possibly the mycotoxins produced are ensiled with the plant material (Wilkinson, 2005; Mansfield and Kuldau, 2007).

Molds are also crucial to the aerobic degradation of silage that occurs during feeding period. The most common molds found in silages belong to the *genera Penicilium, Geotrichum, Aspergillus, Mucor, Fusarium, Absidia, Scopulariopsis, Monascus, Bysochlamys, Arthrinium,* and *Trichoderma* (McDonald et al., 1991).

They are on average the slowest growing microorganisms compared to other microorganisms in silage. They rarely occur in significant or sufficient populations to affect measurements of silage quality until they undergo significant aerobic degradation by yeasts and various aerobic bacteria. Thus, their presence visually indicates silage that is significantly lower quality than the product at ensiling (Muck, 2010). While the total number of molds in silage is not a reliable indicator of mycotoxins, high numbers are frequently linked to silages that have aerobically spoiled (Kung et al., 2018). Since molds are generally aerobic microorganisms, their growth in silage occurs close to the surface layer that is not well sealed or well compacted.

Phytogenic Additives

The use of plant extracts in livestock and poultry nutrition as substitutes for prohibited additives like antibiotics has received more attention. For many years, there have been additives available to improve the fermentation of silage. Producers have had access to an extensive range of silage additives that aid in the preservation of forage (Muck et al., 2018).

Biomolecules, such as phytochemicals found primarily in food, are classified as functional or bioactive components because they can alter one or more metabolic processes in the human body to enhance health and well-being (Abuajah et al., 2015).

Phytochemicals have been used for years in the treatment of various diseases in animals and humans. In addition to the treatment of diseases, these plants are used as performance enhancers in animal feed. In addition, they have antibacterial, antioxidant, anticarcinogenic, antifungal, analgesic, insecticidal and anticoccidial properties. The bioactive components contained in phytochemicals can compete with synthetic drugs and are also used as performance and efficiency enhancing feed additives in animal feeds as well as treatment of diseases. In addition, the majority of phytochemicals have no residue risk in animal products (Tipu et al., 2006). What makes phytogenic plants valuable are the bioactive compounds they contain, and these are called "secondary metabolites". They are organic compounds synthesized by plants but do not have any direct role in the growth and development of plants and are mostly produced for defense against herbivores, microorganisms, and ecological variations (Wink, 1988).

Today, phytogenic additives are used in the animal production chain for a wide variety of purposes such as improving feed palatability, increasing feed consumption, improving animal welfare, animal resistance and health status, preventing and treating diseases, increasing carcass yield, and improving its properties.

Phytogenic feed additives, also called phytobiotics, are a group of natural growth promoters derived from spices and various plants, used as non-antibiotic growth promoters or feed additives. Phytogenics are added to the overall ration and can affect the protein and energy from all the different feed components (feeds, grains, meals, etc.). There are many types of phytogenics and some of the most effective for feed efficiency are:

Spices: Spices have been shown to increase nutrient intake of dairy cows in different trials, and spices have been associated with positively affecting rumen pH by increasing the natural buffer resulting from salivary secretion.

Tannins: Complex polyphenol compounds of plants. Their types and concentrations can vary a lot according to the extraction. Their nature allows binding, especially with protein, but the quantity and quality of the extract is crucial to ensure adequate binding in the rumen and release in the intestine.

Essential oils: There are several families of essential oils with different modes of action. Essential oils are concentrated types of phytogenics containing the most vital elements of plants. In the literature, these compounds have shown antimicrobial activities used to alter secure, increase nutrient digestibility, ruminal fermentations of protein and energy, and improve digestion, and improve productivity. Each active ingredient can exert its antibacterial and antifungal effects independently or synergistically. Therefore, different essential oils have different mechanisms of antibacterial and antifungal action (Evangelista et al., 2022).

Adding an appropriate amount of plant essential oil to reduce the growth and proliferation of harmful microorganisms in silage in the early stages can improve the quality of silage to prevent the growth and proliferation of harmful microorganisms (Chen, et al, 2023).

Leaves: In recent years, the fermentation of leaves used as botanical-based phytogenic feed additives and their presentation to animals has been on the agenda. Thus, it is aimed to overcome the problems related to the high fiber and antinutritional factors contained in the leaves. This is done not only to increase the incorporation levels of leaves into silages, but also to make more use of their content of biologically active compounds due to their effects on improving animal health, enhancing performance, and adding functionality to their products. Live microbes and the metabolites they produce during fermentation can also be very important in enhancing the quality of silages and subsequently their suitability for use as ruminant feed, in addition to the role that bioactive compounds in plant leaves play in this regard. A growing number of studies have shown that phytogenic additives have significant antibacterial and antifungal activity in silages.

The effects of different types of phytogenic additives on silage microbiology are presented in Table 1.

Table 1. Examples of phytogenic additives for silages

Type of phytogenic additives	Levels	Effect on silage microflora	References
A blend of essential plant oils (EO)	Supplemented with 0, 40, or 80 mg of whole-corn silage	The EO did not affect the yeasts, molds, LAB, and Enterobacteria	Kung et al., 2008.
Cinnamon leaf (CIN), oregano (ORE), and sweet orange oils (SO)	Treated with CIN, ORE or SO 37.5, 75 and 120 mg kg^{-1} DM of barley silage	LAB and mold unchanged, yeast decreased with the addition of 120 mg ORE. Total flora decreased in all groups of SO, and in the 75	Chaves et al., 2012.

		and 120 mg groups of CIN and ORE.	
Honey locust pods	Applied at 0, 20, 40, 60, 80 and 100 g/kg DM levels to alfalfa silage	LAB and yeast increased whereas mold decreased.	Canbolat et al., 2013.
Oregano (ORE) and cinnamon (CIN) essential oils	Added to 400 mg/kg CIN, ORE, and ORECIN of field pea silages	After 7 days of aerobic exposure, the number of molds in OA, ORE, and CIN decreased.	Soycan Önenç et al., 2015.
Thymol, eugenol, cinnamaldehyde, capsaicin, carvacrol	Added to 0, 50, 500, and 2000 mg kg−1 of ryegrass	2000 mg thymol, eugenol treatment decreased LAB, no change in clostridia in all groups.	Foskolos et al., 2016.
Dried thyme pulp (DTP)	Supplemented with DTP at 0, 0.5, 1.0, 3.0, and 5.0% level of first-cut meadow grass and alfalfa silage	In both silages, Enterobacter, Clostridia, yeast, mold decreased, LAB did not change in meadow grass silages, but LAB increased in the 5% DTP added group.	Aksu et al., 2017.
Cumin essential oil (CEO)	Supplemented with 300 and 500 mg/kg CEO of alfalfa silage	LAB count increased, Enterobacter and mold were not found, yeast decreased.	Turan and Önenç, 2018.
Moringa oleifera leaves	Added to the at alfalfa and stylo silages levels of 0, 25, and 50 %.	Inclusion of 50 % resulted in higher LAB, and lower Enterobacter and Clostridium abundance.	Wang et al, 2019a.
Lavender flowers	Added to alfalfa silage at 0 %, 0.5, 1, 1.5 and 2	Enterobacter, *Listeria* spp, sulphide reducing anaerobes and yeasts were not found.	Arslan Duru, 2019a.
Lavender (flowers and stem)	Supplemented to alfalfa silage at 0 %, 0.5, 1, 1.5 and 2	Enterobacter, *Listeria* spp, sulphide reducing anaerobes and yeasts were not found. Mold decreased while LAB increased.	Arslan Duru, 2019b.

Oregano and cumin essential oils	Incorporated at alfalfa silages at levels of 650 mg/kg	Total mesophilic aerobic bacteria, Enterobacter, yeast, and mold counts was decreased, while LAB were increased.	Soycan Önenç et al., 2019.
Cumin and Cinnamon Essential Oils	Added to alfalfa silages	LAB increased, while yeast and mold decreased.	Soycan Önenç and Turgud, 2019.
Lemongrass essential oil (LO)	Added to 1, 2, and 3 mL/kg of sugarcane silages	Inclusion of 3 mL LO reduced yeast and mold growth.	Júnior et al., 2020.
Moringa oleifera leaves	Included at 0, 25 and 50% rice straw silages	Treatment increased the abundance of *Lactobacillus*	He et al., 2020.
Lavender Pomace (LP)	Added to alfalfa silage at 0 %, 0.5, 1, 2.5 and 5	LP did not have any effect LAB, but *Listeria* spp were not detect, and sulphite reducing anaerobes, yeast and Enterobacter were below the detection limit.	Arslan Duru, 2020.
Residues of astragalus and hawthorn	Added to alfalfa silage at 1, 1.5, 2, 2.5 and 3 % levels	Showed inhibiting ability of Clostridium, Enterobacter and fungi.	Ni et al., 2020.
Oregano essential oil (OEO)	Sprayed with 10, and 20 ml/75 cm^2 OEO for sugar beet pulp silage	The OEO spraying reduced LAB, total live bacteria, and yeast contents. Mold content did not change.	Çayıroğlu et al., 2020.
Cumin essential oil	Added to vetch-oat silages at 0, 200, 300 and 500 mg/kg levels	LAB increased, and yeast and mold decreased.	Akıncı and Soycan Önenç, 2021.
Amomum villosum essential oil	Supplemented to paper mulberry silage	*Lactococcus, Levilactobacillus* and *Lentilactobacillus* increased but *Stenotrophomonas* decreased.	Li et al., 2022a

Rhubarb stalk	Treated to alfalfa silage	Restricted the growth of yeasts and promoted lactic acid production.	Li et al., 2022b
Herbal residues (36 kind)	Added to alfalfa silage	Stimulation of desired *Lactobacillus* species and inhibited undesirable microbes.	Li et al., 2022c.
Dried mulberry pulp (DMP)	Supplemented with DMP at 0, 2.5, 5, 7.5, 10, 12.5 and 15 of alfalfa silage	Mold was not observed, and yeast increased.	Sengul et al., 2022.
Mugwort residue MR)	Added to alfalfa silage at 460 g alfalfa + 40 g MR	LAB was no change, but *Coliform* bacteria and yeast were decreased.	Guo et al., 2023.
Alecrim Pimenta nanoformulated thymol essential oil (NTO)	Treated with 62% and 100 % NTO of sorghum and paiaguás grass silages	*Clostridium* and *Lactobacillus* counts decreased with nanoformulated thymol.	Sousa et al., 2023.

CONCLUSION

Ensiling is used worldwide as a simple and effective way of preserving feed for livestock by acidifying roughages by natural lactic acid fermentation and then storing them for long periods without spoilage Ensiling is a common way to preserve moist forages with lower weather risk and harvest loss. Feed additives are an important part of the strategy to achieve the goal of safe feed, sustainable manufacturing, and healthy food. Phytogenics play a key role in the future profitable and sustainable production of feed. It also typically leads to higher mixing efficiency, reduced animal weeding activity, and shorter bunk-staying times. The use of phytogenics, either singly or in mixtures, can be part of a global approach to improving silage quality.

REFERENCES

Abuajah, C.I., Ogbonna, A.C., Osuji, C.M. (2015). Functional components and medicinal properties of food: a review. Journal of food science and technology, 52: 2522-2529.

Akinci, Y., Önenç, S.S. (2021). The effect of cumin essential oil on the fermentation quality, aerobic stability, and in vitro digestibility of vetch-oat silages. Ege Üniversitesi Ziraat Fakültesi Dergisi, 58(2): 217-228.

Aksu, T., Denek, N., SS, A., Savrunlu, M., Özkaya, S. (2017). Effect of dried thyme pulp (*Tymbra Spicat*a L. spicata) on fermantation quality and in vitro organic matter digestibility of meadow grass and alfalfa silages. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 23(2): 211-217.

Arslan Duru, A. (2019a). Effects of Lavender (*Lavandula angustifolia*) augmentation of alfalfa silages. South African Journal of Animal Science. 49 (6): 1008-1012.

Arslan Duru, A. (2019b). Lavanta (*Lavandula angustifolia*) Katkısının Yonca Silajlarının Kalitesi Üzerine Etkisi. Uluslararası Mühendislik Araştırma ve Geliştirme Dergisi. UMAGD, 11 (2): 426-431.

Arslan Duru, A. (2020). The Effect of Lavender Pomace on Chemical Composition, Microbiological and Some Fermentation Characteristics of Alfalfa Silages. European International Journal of Science and Technology. 9(2): 1-8.

Basmacıoğlu, H., Ergül, M. (2002). Silaj mikrobiyolojisi. Hayvansal Üretim, 43(1): 12-24.

Canbolat, Ö., Yıldırım, H. K., Filya, İ. (2013). Yonca silajlarında katkı maddesi olarak gladiçya meyvelerinin (*Gleditsia Triacanthos*) kullanılma olanakları. Kafkas Univ Vet Fak Derg. 19 (2): 291-297.

Carr, F.J., Chill, D., Maida, N. (2002). The lactic acid bacteria: A literature survey. Critical Reviews in Microbiology, 28: 281-370.

Chaves, A. V., Baah, J., Wang, Y., McAllister, T. A., Benchaar, C. (2012). Effects of cinnamon leaf, oregano and sweet orange essential oils on fermentation and aerobic stability of barley silage. Journal of the Science of Food and Agriculture, 92(4): 906-915.

Chen, L., Li, X., Wang, Y., Guo, Z., Wang, G., Zhang, Y. (2023). The performance of plant essential oils against lactic acid bacteria and adverse microorganisms in silage production. Frontiers in Plant Science, 14.

Cui, X., Yang, Y., Zhang, M., Jiao, F., Gan, T., Lin, Z., Huang, Y., Wang, H., Liu, S., Bao, C., Su, C., Qian, Y. (2022). Optimized ensiling conditions and microbial community in mulberry leaves silage with inoculants. Frontiers in Microbiology, 13, 813363.

Çayıroğlu, H., Filik, G., Coşkun, İ., Filik, A. G., Çayan, H., Şahin, A. (2020). Spraying opened sugar beet pulp silage with oregano essential oil helps to sustain quality and stability. South African Journal of Animal Science, 50(1): 9-16.

Dogi, C.A., Fochesato, A., Armando, R., Pribull, B., de Souza, M.M.S., da Silva Coelho, I., Araújo de Melo, D., Dalcero, A. and Cavaglieri, L. (2013). Selection of lactic acid bacteria to promote an efficient silage fermentation capable of inhibiting the activity of *Aspergillus parasiticus* and *Fusarium gramineraum* and mycotoxin production. Journal of applied microbiology, 114(6):1650-1660.

Driehuis, F., Odue Elferink, S.J.W.H. (2000). The impact of quality of silage on animal health and food safety: A review. Veterinary Qarterly, 22(4): 212-216.

Evangelista, A. G., Corrêa, J. A. F., Pinto, A. C. S. M., and Luciano, F. B. (2022). The impact of essential oils on antibiotic use in animal production regarding antimicrobial resistance–a review. Critic. Rev. Food Sci. Nutrit. 62 (19): 5267–5283.

Filya, İ. (2001). Silaj fermantasyonu. Atatürk Üniv. Ziraat Fak. Dergisi, 32(1), 87-93.

Foskolos, A., Cavini, S., Ferret, A., Calsamiglia, S. (2016). Effects of essential oil compounds addition on ryegrass silage protein degradation. Canadian Journal of Animal Science, 96 (2): 100-103.

Guo, L., Wang, X., Chen, H., Li, X., Xiong, Y., Zhou, H., Xu, G., Yang, F., Ni, K. (2023). Exploring the fermentation quality, bacterial community and metabolites of alfalfa ensiled with mugwort residues and *Lactiplantibacillus pentosus*. Chemical and Biological Technologies in Agriculture, 10(1): 107.

He, L., Zhou, W., Xing, Y., Pian, R., Chen, X., Zhang, Q. (2020). Improving the quality of rice straw silage with *Moringa oleifera* leaves and propionic acid: Fermentation, nutrition, aerobic stability and microbial communities. Bioresource technology, 299: 122579.

Henderson, A.R., McDonald, P., Woolford, M.K. (1972). Chemical changes and losses during the ensilage of wilted grass treated with formic acid. Journal Science Food Agriculture, 23:1079-1087.

Júnior, R. C., Capucho, E., Garcia, T.M., Del Valle, T.A., Campana, M., Zilio, E.M.C., Azavedo, E.B., Morais, J. P. (2020). Lemongrass essential oil in sugarcane silage: Fermentative profile, losses, chemical composition, and aerobic stability. Animal Feed Science and Technology, 260: 114371.

Kung Jr, L., Williams, P., Schmidt, R.J., Hu, W. (2008). A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. Journal of Dairy Science, 91(12): 4793-4800.

Kung Jr, L., Shaver, R. D., Grant, R. J., Schmidt, R. J. (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. Journal of dairy Science, 101(5): 4020-4033.

Li, M., Fan, X., Cheng, Q., Chen, Y., Long, J., Lei, Y., Li, P., Chen, C. (2022a). Effect of *Amomum villosum* essential oil as an additive on the chemical composition, fermentation quality, and bacterial community of paper mulberry silage. Frontiers in Microbiology, 13, 951958.

Li, P., Zhao, W., Yan, L., Chen, L., Chen, Y., Gou, W., Cheng, Q., Chen, C. (2022b). Inclusion of abandoned rhubarb stalk enhanced anaerobic fermentation of alfalfa on the Qinghai Tibetan Plateau. Bioresource Technology, 347, 126347.

Li, X., Chen, F., Wang, X., Xiong, Y., Liu, Z., Lin, Y., Ni, K., Yang, F. (2022c). Innovative utilization of herbal residues: Exploring the diversity of mechanisms beneficial to regulate anaerobic fermentation of alfalfa. Bioresource Technology, 360, 127429.

Lin, C., Bolsen, K.K., Brent, B.E., Fung, D.Y. (1992). Epiphytic lactic acid bacteria succession during the pre-ensiling and ensiling periods of alfalfa and maize. Journal of Applied Bacteriology, 73(5): 375-387.

Lin, H., Lin, S., Awasthi, M. K., Wang, Y., Xu, P. (2021). Exploring the bacterial community and fermentation characteristics during silage fermentation of abandoned fresh tea leaves. Chemosphere, 283, 131234.

Lv, H., Pian, R., Xing, Y., Zhou, W., Yang, F., Chen, X., Zhang, Q. (2020). Effects of citric acid on fermentation characteristics and bacterial diversity of *Amomum villosum* silage. Bioresource Technology, 307, 123290.

Mansfield, M., Kuldau, G. (2007). Microbiological and molecular determination of mycobiota in fresh and ensiled maize silage. Mycologia 99: 269–278

McDonald, P. (1981). The Biochemistry of Silage. John Wiley & Sons. Chalcome Publications, 226 pp.

McDonald, P., Henderson, A.R., Heron, S.J.E. (1991). The Biochemistry of Silage. Chalcombe Publications, 13 Highwoods Drive, Marlow Bottom. Marlow, Bucks, UK.

Merry, R.J., Davies, R.D. (1999). Propionibacteria and their role in the biological control of aerobic spoilage in silage. Lait, 79: 149-164.

Muck, R.E. (2010). Silage microbiology and its control through additives. Bras. Zootec., 39:183-191.

Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C., Kung Jr, L. (2018). Silage review: Recent advances and future uses of silage additives. Journal of Dairy Science, 101(5): 3980-4000.

Ni, K., Wang, X., Lu, Y., Guo, L., Li, X., Yang, F. (2020). Exploring the silage quality of alfalfa ensiled with the residues of astragalus and hawthorn. Bioresource Technology, 297: 122249.

Ogunade, I.M., Martinez-Tuppia, C., Queiroz, O.C.M., Jiang, Y., Drouin, P., Wu, F., Vyas, D., Adesogan, A.T. (2018). Silage review: Mycotoxins in silage: Occurrence, effects, prevention, and mitigation. Journal of dairy science, 101(5): 4034-4059.

Pahlow, G., Muck, R.E., Driehuis, F., Oude Elferink, S J.W.H., Spoelstra, S.F. (2003). Microbiology of ensiling. Silage science and technology. 42: 31-93.

Sengul, Ö., Sengul, A., Kokten, K., Cacan, E., Beyzi, S. (2022). Possibilities of using dried mulberry pulp as an additive in alfalfa silage. Medycyna Weterynaryjna-Veterinary Medicine-Science and Practice, 78(7): 347-350.

Sousa, B.M.D.L., Santos, S. D. J., Backes, A. A., Silva, C.M., Fagundes, J.L., Blank, A.F., Santos Filho, J.R.D. (2023). 'Alecrim Pimenta' nanoformulated essential oil (*Lippia sidoides*) as additive in consortium silages. Ciência Animal Brasileira, 24.

Soycan-Önenç, S., Koc, F., Coşkuntuna, L., Özdüven, M. L., Gümüş, T. (2015). The effect of oregano and cinnamon essential oils on fermentation quality and aerobic stability of field pea silages. Asian-Australasian journal of animal sciences, 28(9): 1281.

Soycan Onenc, S., Korkmaz Turgud, F., Turan Uçman, A. (2019). Effects of oregano and cumin essential oils on fermentation quality, aerobic stability and in vitro metabolic energy contents of alfalfa silages. J. Anim. Prod, 60(2): 117-123.

Soycan Onenc, S.S., Turgud, F.K. (2019). Effect of oregano, cumin and cinnamon essential oils on fermentation quality in alfalfa silages. Ege Üniversitesi Ziraat Fakültesi Dergisi, 56(3): 367-373.

Spoelstra, S.F., Courtin, M.G., Van Beers, J.A.C. (1988). Acetic acid bacteria can initiate aerobic deterioration of whole crop maize silage. The Journal of Agricultural Science, 111(1): 127-132.

Spoelstra, S.F., Hindle, V.A. (1989). Influence of wilting on chemical and microbial parameters of grass relevant to ensiling. Netherlands Journal of Agricultural Science, 37(4): 355-364.

Tipu, M.A., Akhtar, M.S., Anjum, M.I., Raja, M.L. (2006). New dimension of medicinal plants as animal feed. Pakistan Veterinary Journal, 26 (3): 144-148.

Turan, A., Önenç, S. S. (2018). Effect of cumin essential oil usage on fermentation quality, aerobic stability and in vitro digetibility of alfalfa silage. Asian-Australasian Journal of Animal Sciences, 31(8): 1252.

Xue, Z., Mu, L., Cai, M., Zhang, Y., Wanapat, M., Huang, B. (2020). Effect of using banana by-products and other agricultural residues for beef cattle in southern China. Trop. Anim. Health Prod. 52: 489–496.

Wang, C., He, L., Xing, Y., Zhou, W., Yang, F., Chen, X., Zhang, Q. (2019a). Fermentation quality and microbial community of alfalfa and stylo silage mixed with *Moringa oleifera* leaves, Bioresource Technology, (284): 240-247.

Wang, C., He, L., Xing, Y., Zhou, W., Yang, F., Chen, X., Zhang, Q., (2019b). Effects of mixing *Neolamarckia cadamba* leaves on fermentation quality, microbial community of high moisture alfalfa and stylo silage. Microbial Biotechnology 12 (5): 869–878.

Wang, Y., Chen, X., Wang, C., He, L., Zhou, W., Yang, F., Zhang, Q. (2019c). The bacterial community and fermentation quality of mulberry (*Morus alba*) leaf silage with or without *Lactobacillus casei* and sucrose. Bioresource technology, 293, 122059.

Weinberg, Z.G., Muck, R.E. (1996). New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiology Reviews, 19 (1): 53–68.

Wilkinson, J.M. (2005). Silage. Chalcombe Publications, London.

Wink, M. (1988). Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theoretical and Applied Genetics, 75: 225-233.

Woolford, M.K. (1984). The antimicrobial spectra of organic compounds with respect to their potential as hay preservatives. Grass and Forage Science, 39(1): 75-79.

Woolford, M.K., Honig, H., Fenlon, J.S., Davenport, R.R. (1979). Investigations on the aerobic decomposition in silage using a laboratory method. 3. Microbiological, physical and chemical changes during aerobic decomposition in fresh and prewilted grass silage. Wirtschaftseigene Futter. 24:125-3.

CHAPTER 3

METHODS FOR TRANSCRIPTOME ANALYSIS IN THE CONTEXT OF PLANT-MICROORGANISM INTERACTIONS

Barış KAKİ[1]

DOI: https://dx.doi.org/10.5281/zenodo.10450465

---

[1] Department of Econometrics, Faculty of Economics and Administrative Sciences Uşak University, Uşak, Turkiye **bariskaki@gmail.com** Orchid ID 0000-0002-5836-5438

INTRODUCTION

The highly complex and multifaceted nature of the plant microorganism relationship is vital for plant growth, health, and ecosystem maintenance. Some of the key aspects of these relationships are listed below.

1. Symbiotic relationships such as those between Rhizobium bacteria and mycorrhizae (fungi) and plants
2. Plant pathogen relationships that occur when certain bacteria, phytoplasmas, fungi, viruses, and viroid cause diseases in plants
3. Relationships between antagonistic bacteria and fungi in terms of their natural protective effects on plants.
4. Ensuring the sustainability of the nutrient cycle as decomposing microorganisms break down organic matter.
5. Contribution of endophytic microorganisms to plant defense mechanisms against pathogens and some environmental conditions

These examples illustrate the diverse interactions between plants and microorganisms. These relationships are an important topic of research and understanding in the fields of agriculture, ecology, and plant health (Karapire and Özgönen, 2013; Kumar and Nautiyal 2023).

Interactions between plants and microorganisms are multidimensional and complex. The main factors that make this interaction complex can be listed as follows:

1. Genetic diversity: both plants and micro-organisms have genetic variation. These variations are both within and between species.
2. Environmental factors: Environmental factors such as climate, soil properties, water quantity, etc. influence the interactions between plants and microorganisms. The same plant species may interact with different microorganisms in a different environment.
3. Time: Plants can have different levels of interaction with organisms at different stages of their development.
4. Types of interactions: Not only symbiotic relationships, but also various types of interactions such as competition, predation (hunting), amensalism (one organism harms another while having no effect on the other) play a role in this system. These different types of interactions increase the complexity of the system.

5. Evolution and adaptation: Plants and microorganisms have adapted to each other over a long evolutionary process. These adaptations have evolved in mutually beneficial ways, especially in symbiotic relationships. This makes these interactions more complex.
6. Communication and chemical signaling: Chemical signals play an important role in interactions between plants and microorganisms. Plants can send signals to microorganisms through root secretions or leaf lesions. These signals allow microorganisms to interact with plants.

The combination of these factors makes the interactions between plants and microorganisms highly complex. This complexity plays an important role in the balanced functioning of ecosystems and the sustainability of plant health (Soylu et al. 2016; Rana et al. 2020; Kumar and Nautiyal, 2023).

To deal with the complexity of plant-microorganism interactions and to understand these interactions, bioinformatics methods offer a range of analytical and informatics tools. Bioinformatics methods provide the ability to analyze large data sets, understand complex relationships and resolve these interactions in a systematic way. These methods can help us better understand the mechanisms underlying plant-microorganism interactions and their importance at the ecosystem level.

Bioinformatics Methods

Bioinformatics is a multidisciplinary field representing the combination of biology and information technology. Bioinformatics uses methods such as computer science and statistics to analyze, store, manage and interpret genetic information, molecular biological data, and other biological information. Key goals in this field include understanding genomics, proteomics, metabolomics, and other types of biological data, modeling the functioning of biological systems, discovering the molecular basis of diseases, and providing information for applications such as drug design. Bioinformatics uses computer algorithms and statistical methods to understand and solve large and complex biological data sets (Lucaciu et al. 2019; Keklik and Özcan, 2022).

In this section, brief descriptive information will be given about commonly used bioinformatics methods that can be used to explain plant-microorganism interactions.

Genomic analysis

Definition: Genomic analysis is a discipline that studies the entire genetic material (genome) of an organism. This includes understanding the structural features of the organism's DNA, gene sequence, number of genes and organization of genes.

Scope: Genomic analysis covers the entire genetic information of an organism. This includes protein-coding genes as well as regulatory regions, repeat sequences and other genomic features.

Tools: Tools used in genomic analysis include DNA sequencing techniques, genome mapping methods and genetic mapping.

Genomic analysis is an important area of research to understand the genetic characteristics of organisms, their evolutionary relationships, genetic diseases and to develop genetic-based applications. This analysis is used to make sense of genomic information on a large scale and to unravel complexities at the genome level (Darel and Képès, 2007; Sakiroglu, 2020).

Transcriptome analysis

Description: Transcriptome analysis is a method used to understand which genes are active and how active an organism is at a given moment. This involves examining how much RNA (mRNA) is produced in a cell.

Scope: Transcriptome analysis focuses on gene expression. It aims to determine which genes are active at a given time in a cell and how much these genes are transcribed.

Tools: Tools used for transcriptome analysis include techniques such as RNA sequencing (RNA-seq), microarray technology and quantitative PCR (qPCR).

Transcriptome analysis is important for understanding how genetic material is organized, which genes are active under which conditions and the role of these genes at the cellular level. This analysis is used to understand the regulation of cellular processes and organismal responses by identifying changes in gene expression (Huber et al. 2008).

Proteomics analysis

Definition: Proteomic analysis is a scientific discipline that involves the analysis of all proteins that an organism or a biological system produces at a given moment. This aims to understand how the organism responds at the cellular level and which proteins are synthesized under which conditions.

Scope: Proteomics analysis goes beyond transcriptome analysis, which refers to a specific region of genetic material (DNA) and includes all proteins in the cell. This is used to understand the diversity, quantity, and function of proteins in a cell.

Tools: The main tools used for proteomics analysis include 2D Gel Electrophoresis: A method based on the separation of proteins according to their isoelectric point. LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry): A high-resolution technique that provides mass and amino acid sequence information of proteins. Gel-Filtration Chromatography: A technique based on the separation of proteins according to their size (Dutt and Lee, 2000; Schmidt et al., 2014).

These methods are used to determine the diversity, quantity, modifications, and interactions of proteins in cells. Proteomics analyses are an important tool for understanding the complexity of biological systems and revealing the role of proteins in these systems (Schmidt et al., 2014).

Metabolomics analysis

Definition: Metabolomics analysis is a field of research that involves the systematic analysis of all metabolites (small molecules, products of biological molecules at the cellular level) that an organism or a biological system produces at a given moment. This aims to understand the results of biochemical reactions in cells and to determine how metabolites vary at the organismal level.

Scope: Metabolomic analysis goes beyond transcriptome analysis, which refers to the expression of genetic material, and proteomic analysis, which refers to protein expression. It is used to understand the cellular functions of the organism, energy production, nutrient uptake, and other biological processes.

Tools: Key tools used for metabolomics analysis include:

NMR (Nuclear Magnetic Resonance): Used to determine the chemical structure and measure the concentration of metabolites.

LC-MS (Liquid Chromatography-Mass Spectrometry): A high-resolution technique, LC-MS is used to identify metabolites in a sample.

GC-MS (Gas Chromatography-Mass Spectrometry): Another mass spectrometry technique used for the separation and identification of metabolites.

Metabolite Microarray: Used to identify a set of metabolites and understand their interactions in the organism.

Metabolomics analyses are used to understand how the physiological state of the organism changes under many factors such as disease state, environmental influences, and genetic factors. These analyses are an important tool to better understand the complexity of biological systems and reveal metabolic events at the cellular level (De Iorio et al., 2008; Issaq and Veenstra, 2019).

Functional genomic analysis

Definition: Functional genomic analysis is a field of research that aims to understand the functionality of genetic material (DNA). This involves studying how genes work under certain conditions, how they participate in cellular processes and how the organism's genetic information is expressed.

Scope: Functional genomic analysis aims to understand genetic processes such as gene expression, gene regulation, the function of gene product proteins and their interactions at the cellular level. This is used to reveal how genetic material functions in a particular organism or cell type.

Tools: Key tools used for functional genomic analysis include:

CRISPR/Cas9: A molecular biology technique used for gene editing and modifying genetic material.

RNA Sequencing (RNA-Seq): Used to determine gene expression and analyze the transcriptome.

ChIP-Seq (Chromatin Immunoprecipitation and Sequencing): A method used to identify gene regulatory regions.

Protein-Protein Interaction Analysis: Used to determine physical interactions between proteins.

Functional Genomics Databases: There are several databases that store genomic data and provide tools for analysis and interpretation.

Functional genomic analyses are important for understanding the role of genetic information at the cellular level and the functionality of the organism. These analyses are used to understand the phenotypic consequences of genetic alterations and adaptation of the organism, to reveal disease mechanisms and to identify genetically driven traits (Balding et al., 2008).

Pathway analysis

Definition: Pathway analysis is an analysis method that aims to understand how genes or proteins in biological systems assemble, interact, and work together along specific pathways or processes. This allows to evaluate and interpret genetic data in the broader biological context.

Scope: Pathway analysis has the perspective of studying how genes or proteins interact with each other, how they are involved in specific biological processes and how these processes are regulated at the genetic level. This is a broad field covering biological pathways such as cellular signaling, metabolism, immunology.

Tools: Key tools used for pathway analysis include:

KEGG (Kyoto Encyclopedia of Genes and Genomes): A database of the organization of genetic information in biological systems along pathways.

GO (Gene Ontology): An ontology system that describes the functions, processes and components of genes and proteins.

Reactome: Another database of genetic pathways covering cellular processes, signaling pathways and metabolic pathways.

IPA (Ingenuity Pathway Analysis): A software tool for analyzing and interpreting genomic data and uncovering biological meaning.

Pathway analysis is used to understand the complexity of biological systems by placing genetic data in a broader context. This analysis is an important tool for determining the functional meaning of genetic information, understanding the interactions of genes and proteins, discovering disease mechanisms, and understanding the regulation of biological processes (Khatri et al. 2012).

Metagenomic analysis

Definition: Metagenomic analysis is a field of research that studies the genetic material of a community of microorganisms present in each environmental sample. This is used to understand the genetic diversity of microorganisms in natural environments, to assess the function of these communities and to reveal the genetic structure of ecosystems.

Scope: Metagenomic analysis includes the genomes of all microorganisms in a sample taken from an environment. This includes the genetic material of bacteria, archaea, viruses, and other microorganisms. Metagenomic studies can be performed in a variety of environments such as seawater, soil, human gut microbiota.

Tools: The main tools used for metagenomic analysis include:

DNA Sequencing Techniques: Used to identify genetic material in metagenomic samples.

Bioinformatics Software: Used to analyze metagenomic data and identify microorganism communities.

Metagenomic Databases: Databases containing the genomes of various microorganisms are used for the interpretation and analysis of metagenomic data.

Metagenomic analysis is important for understanding the structures, diversity, potential functions, and ecological roles of microorganism communities. This provides information on the genetic diversity of microorganisms in natural ecosystems and finds application in many fields such as microbial ecology, environmental microbiology, and health sciences (Kukkar et al., 2022).

Development of Transcriptome Analysis

Transcriptome analysis represents the evolution of a set of techniques and methods used to determine gene expression and understand genetic activity within the cell. This evolution initially started with techniques such as Northern blotting, which were limited in resolution and number of genes. The development of DNA microarray technology in the late 1990s made it possible to measure the expression of thousands of genes simultaneously, but with limitations of focusing on specific gene sets.

By the early 2000s, the development of RNA sequencing (RNA-Seq) technology put transcriptome analysis into a broader perspective. RNA-Seq went beyond focusing on specific genes by sequencing entire transcripts, providing high resolution, sensitivity, and genetic coverage. Then came the ability to determine gene expression at the single cell level, allowing a more detailed understanding of different cell types and gene expression profiles within heterogeneous cell populations.

Evolving transcriptome analysis has increased the ability to process and interpret large data sets. Specialized databases and analysis tools have been introduced to understand and evaluate these large amounts of data. Recently, technologies such as artificial intelligence and machine learning have started to play an important role in transcript analysis.

These developments have made transcriptome analysis more sensitive, comprehensive and a powerful tool for biological understanding. The evolution of techniques has allowed for the continuous improvement of methods for determining gene expression and a deeper understanding of genetic information (Bayrak and Ünaltuna, 2011; Zhang, 2019).

Transcriptome Analysis as a Tool to Explain Plant-Microorganism Interaction

This section provides examples of some of the studies that have used transcriptomics to reveal the molecular mechanisms shaping the dynamic relationships between plants and microorganisms.

Symbiotic relationships

In their study, Kawaguchi and Minamisawa (2010) extensively explored the genome sequences of model legumes and the corresponding genetic markers. To enhance accessibility to this valuable information, the L. japonicus genome database (*miyakogusa.jp*) and *M. truncatula* sequencing resources have been meticulously developed. These resources aim to provide comprehensive insights into the genomes and markers of *L. japonicus* and *M. truncatula*, significantly aiding the identification of causal genes related to symbiotic mutants in model legumes.

Chen et al. (2022) conducted a study delving into the intricate interactions between plants and endophytes. Their focus on endophytes, naturally occurring microbes that inhabit plants without causing apparent symptoms, reveals their multifaceted benefits, including promoting plant growth, increasing metabolic capabilities, and enhancing stress resistance. Employing multi-omics technologies, including transcriptomics, the study sheds light on the mechanisms underlying these interactions by comparing endophyte-free (E-) and endophyte-inhabited (E+) plants.

Singh et al. (2021) concentrated on insect-plant interactions, utilizing transcriptomics tools to investigate gene expression. Although the primary focus is on insects, the study provides insights into plant responses at the transcriptome level, offering a glimpse into the complex dynamics between plants and their associated microorganisms.

In the realm of plant-virus interactions, Zanardo et al. (2019) utilized RNA-seq techniques to analyze gene expression patterns, unraveling molecular mechanisms in both compatible and incompatible interactions within the plant-virus pathosystem.

In addition to these studies, Hu et al. (2023) explored interactions between plants in the rhizosphere, emphasizing the role of signaling molecules that vary across different stages of plant development. Transcriptome analysis played a pivotal role in uncovering the underlying mechanisms of these interactions.

Moradi et al. (2021) contributed to the discourse by discussing the relationship between plants and microbes in the rhizosphere. They elucidated how plant growth-promoting rhizobacteria (PGPR) colonize plant roots, inducing beneficial effects such as increased overall plant growth, disease resistance, and enhanced tolerance to abiotic stresses. The authors emphasized the utility of transcriptomics analysis as an ideal technique to comprehend the intricate dynamics of plant-microbe relationships and the contributing factors.

Pathogen-plant relationships

Gao et al. (2022) investigated how plants respond to phosphate starvation and chilling stress, which are two common abiotic stresses that affect plant growth and development. They used RNA-seq to analyze the transcriptome changes in Arabidopsis thaliana under different combinations of these stresses. They found that phosphate starvation and chilling stress induced different sets of genes and pathways, and that some genes involved in photosynthesis and iron uptake were upregulated under both stresses. They also identified some candidate genes that may play important roles in plant adaptation to these stresses.

Kang et al. (2020) studied the molecular interactions between alfalfa (*Medicago sativa L.*) and its rhizobial symbionts (*Ensifer meliloti*) at the below-species level. They used comparative RNA-seq to examine the gene expression patterns of four alfalfa cultivars inoculated with two *E. meliloti* strains that differed in symbiotic performance and phylogenetic relatedness. They found that alfalfa expressed distinct sets of genes in response to different rhizobial strains, and that some of the most significant genes were involved in flavonoid biosynthesis, plant-pathogen interaction, and nodulation. They also observed a positive correlation between plant transcriptomes and rhizobial phylogenetic distances.

Biniaz et al. (2022) performed a transcriptome meta-analysis to identify common and specific responses of Arabidopsis to various pathogens, such as bacteria, fungi, oomycetes, and viruses. They integrated the data from 63 RNA-seq studies and applied different statistical methods to detect differentially expressed genes and pathways. They found that Arabidopsis activated a core set of genes and pathways in response to all pathogens, such as defense-related genes, hormone signaling, and cell wall modification. They also found that Arabidopsis modulated specific genes and pathways depending on the type, lifestyle, and infection stage of the pathogens.

Continuing with the exploration of plant-pathogen relationships, Nobori et al. (2018) analyzed the transcriptome landscape of a bacterial pathogen (*Pseudomonas syringae pv. tomato* DC3000) under plant immunity. They used RNA-seq to measure the gene expression changes of the bacterium in the leaves of Arabidopsis and tomato after triggering two types of plant immune responses: pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). They found that the bacterium altered its expression of genes involved in metabolism, stress response, virulence, and motility under plant immunity. They also identified some bacterial genes and processes that were specifically responsive to PTI or ETI.

Muchero et al. (2018) identified candidate genes mediating plant-pathogen interactions in a tree (*Populus trichocarpa*). They used a combination of genome-wide association mapping, transcriptomics, and transient expression to study the genetic basis of resistance to a fungal pathogen (*Sphaerulina musiva*) in poplar. They found that a gene encoding a NAC domain-containing protein (PtrWND3A) was associated with resistance to the pathogen and was induced by infection. They also showed that overexpression of PtrWND3A in poplar enhanced resistance to the pathogen and altered the expression of genes involved in lignin biosynthesis and cell wall modification.

Antagonistic microorganisms and plant interactions

Examining the interaction between Pseudomonas fluorescens and chickpea roots, Gupta et al. (2019) found that transcriptome analysis revealed upregulated genes associated with nutrient uptake, stress tolerance, and hormone signaling. The presence of *P. fluorescens* enhanced chickpea growth and conferred resistance against root pathogens.

In a similar vein, Zhang et al. (2021) studied the impact of *Bacillus amyloliquefaciens* on maize transcriptomes, revealing genes involved in defense responses. RNA-seq analysis identified genes involved in defense responses, including jasmonic acid (JA) and salicylic acid (SA) pathways. The endophytic bacteria induced systemic resistance against fungal pathogens, highlighting their potential for sustainable crop protection.

Wu et al. (2022) investigated the protective effects of Streptomyces strains on Arabidopsis under drought stress, with transcriptome analysis revealing upregulated genes related to osmotic regulation, antioxidant defense, and hormone signaling. The endophytic Streptomyces improved plant survival and water use efficiency.

Yang et al. (2023)" examined the interaction between *Bacillus velezensis* and soybean (*Glycine max*) roots. Transcriptomic profiling identified genes associated with nutrient acquisition, stress tolerance, and growth promotion. The endophytic bacteria enhanced soybean growth and protected against soilborne pathogens.

Kang et al. (2020) investigated specific molecular interactions between alfalfa (*Medicago sativa*) and its rhizobial symbionts at the below-species level. They identified 12 rhizobial biotypes of *Ensifer meliloti* that form nitrogen-fixing nodules in alfalfa roots. Comparative RNA-seq analysis revealed differentially expressed genes (DEGs) associated with flavonoid biosynthesis, plant-pathogen interactions, and nodulation. Strong correlations were observed between plant transcriptomes and phylogenetic distances of the rhizobial inoculants.

Zhao et al. (2021) explored the impact of drought and high-temperature stress on rice (*Oryza sativa*) transcriptomes. RNA-seq analysis revealed upregulated genes related to osmotic regulation under drought and heat stress. Transcription factors associated with stress tolerance were also identified, providing insights for crop improvement strategies.

These studies shed light on the protective benefits of antagonistic bacteria and mats for plants, underscoring the significance of transcriptome analysis in deciphering intricate biological interplays.

Decomposing microorganisms and nutrient cycle sustainability

Continuing the exploration of microbial interactions, Coonan et al. (2020) studied microbial detritus and demonstrated its significant contribution to soil organic matter (SOM). Bacterial detritus played a very important role, especially in grasslands. Nutrient availability influenced microbial biomass assimilation and SOM re-metabolism. Carbon transfer to SOM was enhanced by balanced nutrient addition, thus maintaining its stability. Terrestrial biogeochemistry is regulated by both living and dead microorganisms that build and break down the SOM, which contains the largest stock of terrestrial organic carbon and nitrogen. The genetic basis of this vital process has been revealed by transcriptome analysis.

The soil microbiome deciphered through transcriptomics directly influenced nutrient cycling. Understanding microbial communities has become crucial for sustainable agriculture and carbon sequestration (Suman et al., 2023). Transcriptome insights have elucidated the dominant bacterial phylum during composting. These microorganisms have driven nutrient transformations, emphasizing the importance of their genetic activity (Aguilar-Paredes et al., 2023).

To sum up, transcriptome analysis has unveiled the intricate molecular symphony within microorganisms, exerting a profound influence on nutrient cycles and shaping the destiny of organic matter.

Endophytic microorganisms, pathogens, and defense mechanisms of plants

In natural ecosystems, plant-symbiont-pathogen interactions play crucial roles in mitigating abiotic and biotic stresses. Endophytes, naturally occurring microbes that inhabit plants without causing apparent symptoms, emerge as advantageous sources of novel metabolites, growth promoters, and stress resistance in their host plants. These interactions involve complex mechanisms, ranging from colonization to protection against adverse conditions. Transcriptomics and metabolomics provide insights into these intricate processes. By adopting a multi-omics approach, researchers uncover the molecular dialogues between plants and endophytes, enhancing our understanding of recognition, colonization, and their applications in sustainable agriculture and environmental resilience.

Maintaining microbiota stability is critical for phytopathogen biocontrol and grapevine management. The abundance and richness balance between beneficial microorganisms plays a pivotal role. Environmental factors, including soil type, geography, and climate, significantly influence microbiota stability. These endophytic microorganisms, directly or indirectly, synthesize plant growth regulators, secondary metabolites, and defense compounds. Their intricate role in maintaining plant health underscores the importance of understanding their genetic activity through transcriptome analysis. By harnessing their potential, we can enhance crop resilience and sustainable agricultural practices.

In conclusion, transcriptome analysis unravels the molecular intricacies of plant-endophyte interactions, exerting influence over nutrient cycling, stress responses, and overall ecosystem health.

CONCLUSION

The synthesis of findings across the reviewed studies underscores the pivotal role of transcriptomics in unraveling the intricacies of plant-microbe interactions. Through the lens of gene expression dynamics, regulatory networks, and functional pathways, transcriptomics serves as a formidable tool, shedding light on the diverse and complex nature of these associations. However, this analytical approach is not without its challenges and limitations, necessitating careful consideration and strategic directions for future investigations.

Methodological Considerations and Challenges:

While transcriptomics provides a powerful means to decipher molecular dialogues, ensuring the reliability of data remains paramount. Rigorous attention must be given to data quality, robust analysis methods, and the integration of complementary omics technologies for a comprehensive understanding.

Recommendations for Future Research:

Integration of Omics Technologies: The synergy of transcriptomics with genomics, proteomics, metabolomics, and epigenomics is advocated to attain a more holistic perspective on plant-microbe interactions.

Expansion to Non-Model Organisms: Broadening the scope by applying transcriptomics to non-model or under-studied plants and microbial species is crucial for enriching our comprehension of the diversity inherent in these interactions.

Advancements in Transcriptomics Tools: Innovations in transcriptomics tools, such as the incorporation of single-cell or spatial transcriptomics, are urged to capture the nuanced heterogeneity characterizing plant-microbe associations.

Validation Through Functional Assays: To elucidate causal genes and mechanisms, the coupling of transcriptomics with functional or phenotypic assays, including gene editing or mutant screening, is advocated for rigorous validation.

Practical Applications of Transcriptomics: The exploration of practical applications and implications of transcriptomics in the realm of plant-microbe interactions is paramount. This includes the development of strategies or products with potential applications in crop improvement, protection, and management.

In conclusion, while transcriptomics stands as a cornerstone in unraveling the molecular intricacies of plant-microbe interactions, a concerted effort towards overcoming challenges and embracing innovative approaches is essential for advancing the field and realizing its full potential.

REFERENCES

Aguilar-Paredes, A., Valdés, G., Araneda, N., Valdebenito, E., Hansen, F., & Nuti, M. (2023). Microbial Community in the Composting Process and Its Positive Impact on the Soil Biota in Sustainable Agriculture. Agronomy, 13(2), 542. https://doi.org/10.3390/agronomy13020542

Akram, S., Ahmed, A., He, P., He, P., Liu, Y., Wu, Y., & He, Y. (2023). Uniting the Role of Endophytic Fungi against Plant Pathogens and Their Interaction. Journal of Fungi, 9(1), 72. https://doi.org/10.3390/jof9010072

Balding, D J., Bishop, M., & Cannings, C. (Eds.). (2008). Handbook of statistical genetics 1. John Wiley & Sons.

Biniaz, Y., Tahmasebi, A., Tahmasebi, A., Albrectsen, B.R., Poczai, P., & Afsharifar, A. (2022). Transcriptome Meta-Analysis Identifies Candidate Hub Genes and Pathways of Pathogen Stress Responses in Arabidopsis thaliana. Biology, 11(8), 1155. https://doi.org/10.3390/biology11081155

Chen, X.L., Sun, M. C., Chong, S.L., Si, J.P., & Wu, L.S. (2022). Transcriptomic and metabolomic approaches deepen our knowledge of plant–endophyte interactions. Frontiers in Plant Science, Volume 12 - 2021 | https://doi.org/10.3389/fpls.2021.700200

Coonan, E.C., Kirkby, C.A., Kirkegaard, J.A., Amidy, M.R., Strong, C.L., & Richardson, A.E. (2020). Microorganisms and nutrient stoichiometry as mediators of soil organic matter dynamics. Nutrient Cycling in Agroecosystems, 117(3), 273-298. https://doi.org/10.1007/s10705-020-10076-8

Dardel, F., & Képès, F. (2007). Bioinformatics: genomics and post-genomics. John Wiley & Sons.

De Iorio, M., Ebbels, T.M., & Stephens, D.A. (2008). Statistical techniques in metabolic profiling. Handbook of statistical genetics, 1, 347-373

Dutt, M.J., & Lee, K.H. (2000). Proteomic analysis. Current opinion in biotechnology, 11(2), 176-179.

Ek-Ramos, M.J., Gomez-Flores, R., Orozco-Flores, A.A., Rodríguez-Padilla, C., González-Ochoa, G., & Tamez-Guerra, P. (2019). Bioactive products from plant-endophytic gram-positive bacteria. Frontiers in microbiology, 10, 463.https://doi.org/10.3389/fmicb.2019.00463

Gao, X., Dong, J., Rasouli, F., Pouya, A.K., Tahir, A.T., & Kang, J. (2022). Transcriptome analysis provides new insights into plants responses under phosphate starvation in association with chilling stress. BMC plant biology, 22(1), 1-14. https://doi.org/10.1186/s12870-021-03381-z

Gupta, G.S., Kumar, A., and Verma, N. (2019). Bacterial homoserine lactones as a nanocomposite fertilizer and defense regulator for chickpeas. Environ. Sci. Nano 6, 1246–1258. doi: 10.1039/c9en00199a

Hu, Y., Tang, F., Zhang, D., Shen, S., Peng, X. (2023). Integrating genome-wide association and transcriptome analysis to provide molecular insights into heterophylly and eco-adaptability in woody plants, Horticulture Research, Volume 10, Issue 11, https://doi.org/10.1093/hr/uhad212

Huber, W., Heydebreck, A.V., & Vingron, M. (2008). Analysis of microarray gene expression data. Handbook of Statistical Genetics 1. John Wiley & Sons.203-230.

Issaq, H.J., & Veenstra, T.D. (Eds.). (2019). Proteomic and metabolomic approaches to biomarker discovery. Academic Press.

Kang, W., Jiang, Z., Chen, Y., Wu, F., Liu, C., Wang, H., & Zhang, X. X. (2020). Plant transcriptome analysis reveals specific molecular interactions between alfalfa and its rhizobial symbionts below the species level. BMC Plant Biology, 20(1), 1-19. https://doi.org/10.1186/s12870-020-02503-3

Karapire, M., & Özgönen, H. (2013). Doğada yararlı mikroorganizmalar arasındaki etkileşimler ve tarımsal üretimde önemi. Türk Bilimsel Derlemeler Dergisi, (2), 149-157.

Kawaguchi, M., Minamisawa, K., (2010). Plant–Microbe Communications for Symbiosis. Plant and Cell Physiology, Volume 51, Issue 9, September 2010, Pages 1377–1380, https://doi.org/10.1093/pcp/pcq125

Keklik, G., & Özcan, B. D. (2022). Multidisipliner Bir Bilim Dalı Olarak Biyoinformatiğe Genel Bir Bakış. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 5(2), 1082-1091.

Khatri, P., Sirota, M., & Butte, A.J. (2012). Ten years of pathway analysis: current approaches and outstanding challenges. PLoS computational biology, 8(2), e1002375.

Komurcu-Bayrak, E., & Erginel-Ünaltuna, N. (2011). Gen anlatımı analiz yöntemlerine genel bakış. Deneysel Tıp Araştırma Enstitüsü Dergisi, 1(2), 28-35.

Kukkar, D., Sharma, P.K., & Kim, K.H. (2022). Recent advances in metagenomic analysis of different ecological niches for enhanced biodegradation of recalcitrant lignocellulosic biomass. Environmental Research, 114369.

Kumar, V., & Nautiyal, C.S. (2023). Endophytes Modulate Plant Genes: Present Status and Future Perspectives. Current Microbiology, 80(11), 353. https://doi.org/10.1007/s00284-023-03466-y

Lucaciu, R., Pelikan, C., Gerner, S. M., Zioutis, C., Köstlbacher, S., Marx, H., & Rattei, T. (2019). A bioinformatics guide to plant microbiome analysis. Frontiers in Plant Science, 10, 1313. https://doi.org/10.3389/fpls.2019.01313

Moradi, S., Khoshru, B., Mitra, D., Mahakur, B., Das Mohapatra, P.K., Lajayer, B.A., Ghorbanpour, M., (2021). Transcriptomics Analyses and the Relationship Between Plant and Plant Growth-Promoting Rhizobacteria (PGPR). In: Pudake, R.N., Sahu, B.B., Kumari, M., Sharma, A.K. (eds) Omics Science for Rhizosphere Biology. Rhizosphere Biology. Springer, Singapore. https://doi.org/10.1007/978-981-16-0889-6_6

Muchero, W., Sondreli, K.L., Chen, J.G., Urbanowicz, B. R., Zhang, J., Singan, V., & LeBoldus, J. M. (2018). Association mapping, transcriptomics, and transient expression identify candidate genes mediating plant–pathogen interactions in a tree. Proceedings of the National Academy of Sciences, 115(45), 11573-11578. doi: 10.1073/pnas.1804428115

Nobori, T., Velásquez, A.C., Wu, J., Kvitko, B.H., Kremer, J.M., Wang, Y., & Tsuda, K. (2018). Transcriptome landscape of a bacterial pathogen under plant immunity. Proceedings of the National Academy of Sciences, 115(13), E3055-E3064. https://doi.org/10.1073/pnas.1800529115

Rana, K.L., Kour, D., Kaur, T., Devi, R., Yadav, A. N., Yadav, N., & Saxena, A. K. (2020). Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. Antonie Van Leeuwenhoek, 113, 1075-1107. https://doi.org/10.1007/s10482-020-01429-y

Sakiroglu, M. (2020). Genomik Analiz İçin Biyoinformatik Yöntemler, Palme Yayıncılık, Barcode: 9786052825419, 245p.

Schmidt, A., Forne, I., & Imhof, A. (2014). Bioinformatic analysis of proteomics data. BMC systems biology, 8(2), 1-7.

Singh, S., Singh, A., Singh, I.K. (2021). Transcriptomics Studies Revealing Enigma of Insect-Plant Interaction. In: Singh, I.K., Singh, A. (eds) Plant-Pest Interactions: From Molecular Mechanisms to Chemical Ecology. Springer, Singapore. https://doi.org/10.1007/978-981-15-2467-7_2

Soylu, S., Sülü, S. M., & Bozkurt, İ. A. (2016). Bitki büyüme düzenleyici ve biyolojik mücadele etmeni olarak bakteriyel endofitler. Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi, 21(1).

Suman, J., Rakshit, A., Ogireddy, S. D., Singh, S., Gupta, C., & Chandrakala, J. (2022). Microbiome as a key player in sustainable agriculture and human health. Frontiers in Soil Science, 2, 821589. https://doi.org/10.3389/fsoil.2022.821589

Wu, X., Wang, Z., Zhang, R., Xu, T., Zhao, J., & Liu, Y. (2022). Diversity of endophytic bacteria in hybrid maize seeds and Bacillus mojavensis J2416-7 may be capable of vertical transmission. Archives of Microbiology, 204(4), 213. https://doi.org/10.1007/s00203-022-02824-x

Yang, Z., Wu, X., Yang, Y., Qu, Y., Xu, J., Wu, D., & Li, Y. (2023). Identification of QTNs, QEIs interactions and genes for isoflavones in soybean seeds. Industrial Crops and Products, 197, 116631. https://doi.org/10.1016/j.indcrop.2023.116631

Zanardo, L.G., de Souza, G.B. & Alves, M.S. (2019). Transcriptomics of plant–virus interactions: a review. Theor. Exp. Plant Physiol. 31, 103–125 https://doi.org/10.1007/s40626-019-00143-z

Zhang, H. (2019, October). The review of transcriptome sequencing: principles, history and advances. In IOP conference series: Earth and environmental science (Vol. 332, No. 4, p. 042003). IOP Publishing.

Zhao, Z., Kou, M., Zhong, R., Xia, C., Christensen, M.J., & Zhang, X. (2021). Transcriptome analysis revealed plant hormone biosynthesis and response pathway modification by Epichloë gansuensis in Achnatherum inebrians under different soil moisture availability. Journal of Fungi, 7(8), 640 https://doi.org/10.3390/jof7080640

CHAPTER 4

SEED BIOPRIMING AND THE ROLE OF MICROBIAL AGENTS

Burcu Begüm KENANOĞLU[1]
Havva DİNLER[2]

DOI: https://dx.doi.org/10.5281/zenodo.10450467

---

[1] Department of Horticulture, Faculty of Agriculture, Uşak University, Uşak, Turkiye burcu.kenanoglu@usak.edu.tr Orcid ID 0000-0001-5307-5194

[2] Department of Plant Protection, Faculty of Agriculture, Uşak University, Uşak, Turkiye havva.dinler@usak.edu.tr Orcid ID 0000-0002-7011-5183

INTRODUCTION

Global warming, population growth, deforestation, drought, and other problems affect plant growth at the seedling stage in the present environment (Meyerson, 2004). As a result, various inner and extrinsic variables that appear to be responsible for seed germination inhibition have been found. The primary reasons for germination limitation are salinity, dryness, soil pH, light, temperature, storms, fires, and other abiotic factors (Humphries et al., 2018). Furthermore, herbivorous species infected with pathogens and other biotic factors decrease seed germination. Moreover, herbivorous species inoculated with pathogens and other biotic factors lead to seed germination inhibition (Doughari, 2015). It is estimated by Pandey et al. (2017) that 40 to 50 percent of crops are lost every year due to various stress factors imposed by various biotic and abiotic variables. The most important and sensitive stage in the life cycle of a plant is seed germination. The seed is a plant embryo covered by a barrier called testa. Compared to cryptograms that cannot produce seeds, phanerogams have longer life span, geographical range and reproductive success. A seed contains all the components necessary for the development and maturation of a young plant, including the embryo, a viable endosperm, and a covering seed coat.

Biopriming is an application with beneficial microorganisms. Generally, it can also be applied for developing seedlings to become more resistant. The researchers coated sweet corn seeds with beneficial bacteria. Biopriming is used to improve seed quality in different species for germination enhancement, sample germination, seed vigour, seed emergence and disease resistance. Biological seed coating is a seed treatment technology that coats the seed surface using microbial inoculants to prevent seed-borne and soil-borne diseases. The effectiveness of microbial inoculants relies on the selection of the appropriate microbial strain, improved adaptability, a longer life span, innovative transporters, as well as additional additions. The formulation has a major effect on microbial survival throughout the product creation, storage, and application rules in the field, as well as the product's efficiency when treated to the plant host and the commercial viability of the application. Seed coating and priming procedures should be established and uniform for every type of field, plantation, and horticultural crops, and should be cost-effective, economical, and acceptable to farmers of all types.

What Is Priming and Effect Mechanisms?

Both biotic and abiotic factors affect crop quality and quantity. As explained by Vittori et al. (2018), quality is defined as a combination of agronomic, organoleptic, and nutritional properties. The kind of soil, in particular its high or low salinity or acidity, drought, contamination, hot or cold temperatures, precipitation, humidity, UV radiation or winds are examples of abiotic characteristics. Adverse signals can cause plants to utilize stored energy to endure stress rather than rely on yield, which can dramatically diminish harvest yield. The biological variables involve bacteria, fungi, and viruses which lead to plant diseases. The seedling quality can be described by many characteristics. Benefits include no diseases or pests, high dry matter, an adequate root system, rapid new root development, a balanced root/shoot ratio, healthy leaves, optimum levels of minerals and carbohydrates and adaptability. Stem length, stem thickness, node distance, seedling fresh and dry weight, and pH and EC values of the growing medium that affect these criteria are important. Low-temperature stress during the early phases of seedling emergence can be a cause of poor seedling emergence uniformity, particularly when limited viable seed batches are used (Mavi and Demir, 2007). Germination and seedling growth are important to the successful development and continued growth potential of plants. Low water potential seed hydration, also known as priming, has frequently been used to shorten the germination period, synchronize emergence, and improve plant development in small seed crops (Heydecker et al., 1975).

The technique of seed priming dates back to 60 AD. There have been several records dating back to the ancient Greeks reporting experiments carried out to promote seed germination (Evenari, 1984). Theophrastus (371-287 BC) observed that seeds soaking with water before sowing germinated rapidly. Gaius confirmed the importance of soaking seeds with water to improve germination (1949-1954). Priming is the process of preparing seeds before planting by treating them to a low external water potential, which allows for pre germinative physiological and biochemical activity while inhibiting germination via various ways (Taylor et al., 1998; Thornton and Powell, 1995). Priming improves seed performance by enhancing the rate and uniformity of germination, resulting in faster and better seedling development (Fig.1). This has been observed in many agricultural seeds (Powell et al., 2000; Warren and

Bennet, 1997). Hydration is an efficient, low-cost, simple, and effective means of enhancing seed germination and seedling growth among the numerous priming procedures (Thornton and Powell, 1992). Furthermore, hydration benefits poor transplant growers and farmers (Harris et al., 1999). Because of the small size of their seeds and slow germination, obtaining early and regular emergence might be difficult in some cases, especially when the seeds are planted deeply. Sub-optimal temperatures can also be the cause of low and variable germination in modules because of poor seed quality. Slow and heterogenity emergence causes differences in plant size and reduces the total quality of the transplant (Cantliffe, 1998; Watkins, 1998).

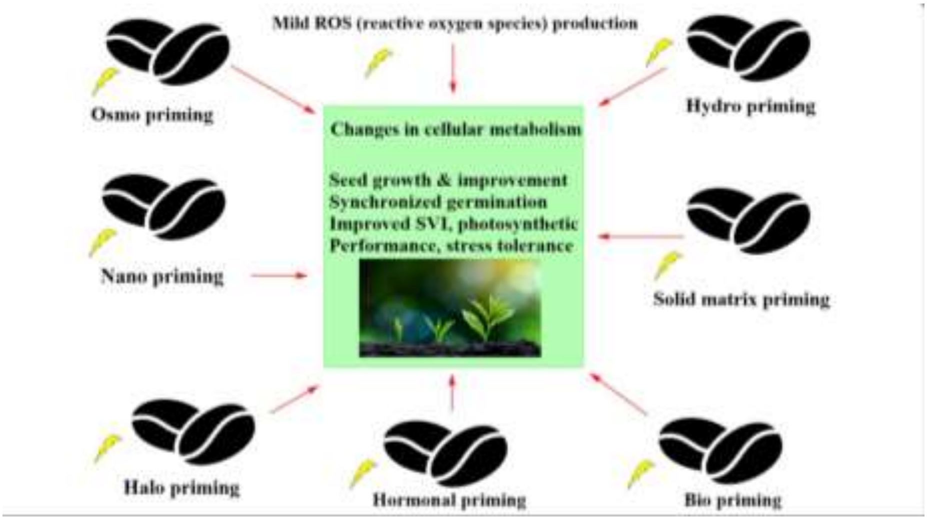


Figure 1. Methods for priming seeds to improve seed germination and physiological state of plants (Nile et al., 2022)

The seed germination process consists of three major phases, namely, (I) seed hydration, (II) activation of metabolic events, and (III) radicle emergence, accompanied by cell elongation. Seed priming is a low-cost easy procedure that improves seedling emergence, promotes uniform stand establishment, increases flowering, and results in a more productive crop (Ullah et al., 2019a; Rehman and Farooq, 2016). Essentially, seed priming means hydrating a seed to a necessary level to activate metabolic activities, but not to cause radicle emergence. Basically, priming techniques can be divided into three phases,

I. In this stage, the seed is being hydrated and absorbing water.

II. After sufficient water has been absorbed, a number of biochemical processes are initiated which then lead to the germination of the seed.

II. During priming, the seed is transported through Phase II before being dried. This occurs before the seed takes root. When temperature and moisture conditions in the field are favorable, this phase can be restarted, and propagation begins in a significantly shorter time.

Types of Priming

Currently, various priming techniques are used, including hydropriming, osmopriming, halopriming, solid matrix priming, hormonal priming, thermopriming, smoke priming, and micronutrient priming. Hydropriming is the most basic method of hydrating seeds while reducing the usage of pesticides. Polyethylene glycol (PEG), mannitol, glycerol, and sucrose are examples of osmotic substances that may be used in osmopriming processes (Parera and Cantliffe, 1994). Hormone priming is a term used to describe priming processes which affect seed physiology by exposing the seed with a hormone solution. In the last few decades, the use of organic substances that improve seed percentage of germination and homogeneous seedling emergence has gained popularity which called organic priming. marigold herbal tea, seaweed, humic acid, grapefruit juice, and fulvic acid have all been shown to be effective in seed treatments (Mavi, 2014). Micronutrient priming is a technique which involves immersing seeds in a nutrient solution rather than pure water to increase seed nutrient content while improving seed quality to get faster germination and seedling growth (Imran et al., 2013). Thermopriming is the process of soaking seeds for extended periods of time in distiled water with extreme temperatures oxygen. As the matrix priming treatment provides a significantly more airy environment than previous priming applications, there is no extra airflow is necessary (McDonald, 2000). Currently, researchers are studying the usefulness of smoke derived from plants as a priming agent. This research aims to reduce seed dormancy, increase seed viability, and improve seedling quality.

What is Biopriming and Its Importance?

This method involves hydrating seeds and treating them with a biocontrol agent, which is a beneficial bacteria or fungus. Bio-priming has lately been used as an alternate strategy for managing various seed-borne and soil-borne diseases (Begum et al., 2010). At moderate (23 °C) and humid circumstances, seeds are moistened followed by inoculation with a bio-control agent, which can be either beneficial bacteria or fungi, for approximately 20 hours. once then, seeds are harvested before roots form (Callan et al., 1990). Seed biopriming is a standard technique for getting a large population of beneficial micro-organisms into the soil, where they can colonise the developing roots of crops. This method has been used effectively in the field for decades and produces better or comparable results to traditional harmful fungicides (Raj et al., 2004). Seed biopriming with microorganisms improves plant growth and development by regulating a variety of biochemical and physiological activities and providing plants with stress tolerance and resistance mechanisms. Biopriming is used to activate certain signalling pathways in the earlier stages of plant phenology, resulting in more rapid plant protection mechanisms. After infection, a second signaling pathway is initiated, which eventually leads to an increase in signal transduction. This results in a rapid and significant enhancement of the protective mechanisms that are already present. Also, seed biopriming has many benefits against chemical treatments, which include having economically viable, quick, sustainable, while offering beneficial features for primed seeds. This technology also allows farmers to maximum productivity with minimal resources, improving their economic position and helping to alleviate the global food crisis. Further studies on seed biopriming technology should be conducted and centered on producing of microbial solutions that are more tailored to specific environmental circumstances.

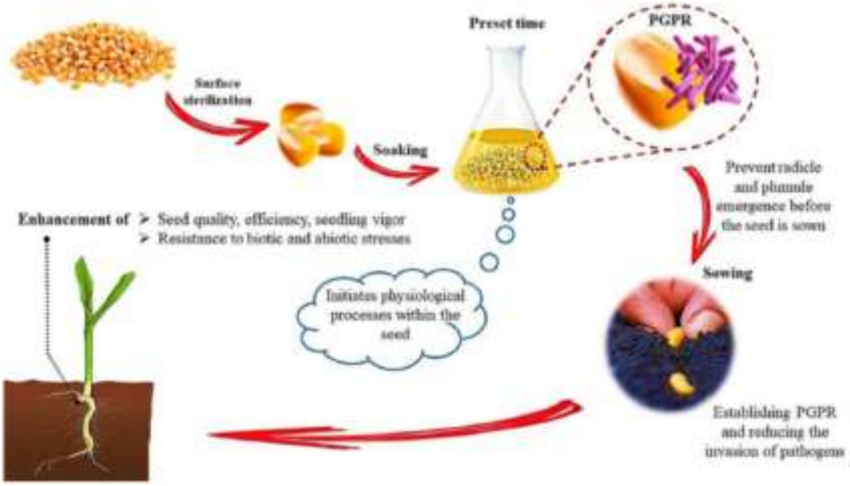


Figure 2. Methods of seed biopriming with PGPR (Mitra et al., 2021)

Biopriming Agents and Efficiencies

Plant roots in the rhizosphere are subjected to different types of microorganisms. Plants have a close association with these organisms, which can be useful or harmful. According to several studies, seed biopriming with beneficial microorganisms found inside or near plant roots enhances germination percentage, seedling vigor, growth potential (Fig. 3). Biopriming agents can either release or encourage the production of phytohormones such as gibberellin, auxin and cytokinin in plants. In addition, they improve plant growth by increasing the supply of minerals such as N, P, K, Fe, and others. Range of fungal or bacterial bio agents viz., Azotobacter (Balasubramanian et al., 2018), Rhizobium, Arthrobacter, Agrobacterium, Azospirillum (Sowmya et al., 2022), Enterobacter (Bhatt et al., 2015), Serratia (Roberts et al., 2016) and Streptomyces (Abbasi et al., 2020), Streptomyces, Bacillus (Kaymak et al. 2009; Song et al. 2017), Burkholderia, Klebsiella, PSB, *Pseudomonas fluorescence*, *Trichoderma viride*, *Trichoderma harzianum* and Vesicular Arbuscular Mycorhiza, whether they are biofertilizer or biopesticide, may be useful as biopriming agents (Deshmukh et al., 2020).

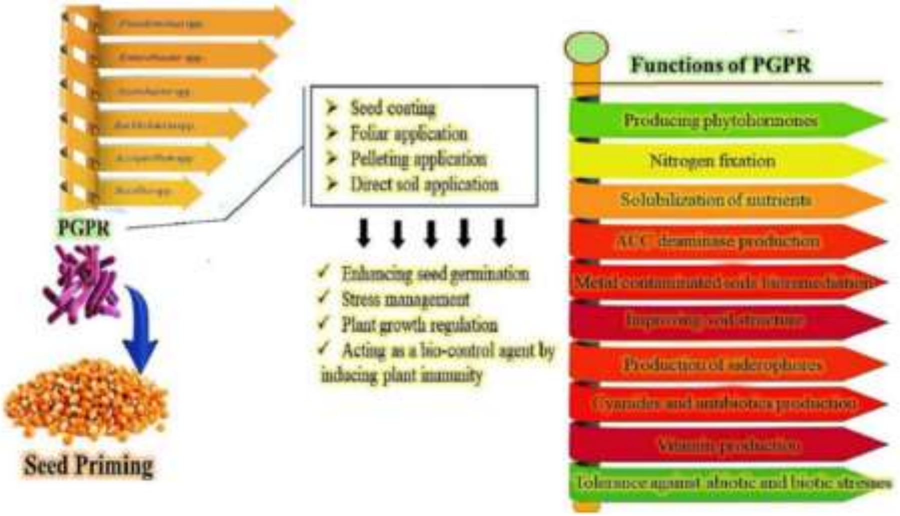


Figure 3. Biopriming with PGPR inoculant triggers the plant growth and health (Mitra et al., 2021)

Biopriming is a global approach to sustainable agriculture which aims to enhance seed viability, seedling vigour, performance and tolerance to biotic and abiotic stresses by limiting the use of chemical treatments. It is associated with an increase in hydrolytic enzyme activities, ROS (reactive oxygen species) detoxifying enzyme activities, changes in endogenous plant hormone levels, and variation gene expression in plants, all of which lead to improved plant growth and resilience to stressful situations. The fundamental mechanism of this method consists of this actions; Positive effects on seedling development/vigor and antibiotic impact on microorganisms that cause disease. The ability of a seed to germinate and generate a strong seedling determines the long-term survival and reproduction of the types of plant (Babu et al., 2015). Using bioagents to prime seeds improves both germination rates and stress resistance in early seedling growth (Fallahi et al., 2011). Biopriming stimulates seed germination generally through phytohormone production and activation. It has been confirmed that biopriming improves the amount of GA and ABA (El-Araby et al., 2006). Homogeneous internal GA content in primed seed may promote synchronous deterioration of the endosperm, embryo, and reserve activation (Sung et al., 2008). By enhancing the ATP-synthesising enzyme system, rapid imbibition process in primed seeds will stimulate adequate activity of the mitochondria (Singh et al., 2003). PGPR used in biopriming stimulates the formation of specific phenolic acids at different stages of growth. Moreover, higher IAA levels in plants enhance the growth of roots, including root number/hairs and area, as well as promoting lateral roots and adventitious development, cell division/differentiation, phyto-pigment biosynthesis, differential metabolites and vascular tissue development (Naik, 2015). Biopriming in plants leads to various biochemical changes, including increased protein production, hormone synthesis, and storage of phenols and flavonoids. These changes contribute to the enhanced growth of the plant. In perennial plants, growth reactions are influenced by nitrogen reserve compounds such as proteins, nitrates, and amino acids (Dhanya, 2014).

Seed priming promotes endosperm weakening and post-priming germination by increasing ethylene synthesis in treated seeds (Chen and Arora, 2013). Also, biopriming is known to activate the mending and restarting of elderly mitochondria, as well as the synthesis with new mitochondria (Sun et al., 2011). Microorganisms in agriculture can reduce mineral usage without sacrificing productivity. Microbial treatments play a key role in this by influencing enzymatic activity in the rhizosphere due to nutrient variations. Additionally, microorganisms have a known defense effect against a range of stresses, justifying their multifunctional use in integrated agriculture. Seed biopriming by microorganisms increased root related properties and provided a vigorous root structure. It improves root to shoot proportion, root dried weight, leaf quantity and size, and chlorophyll levels in crops, in addition to contributing to improved root system biopriming of seeds (Priya et al., 2016). Early flowering formation, enhanced plant height, number of branches, dry pod weight, pods and seeds per plant, seed yields, and 100-seed weight were all results of biopriming (Naik, 2015).

Plants are exposed to many types of stress, mostly biotic and abiotic, and respond to this stress by using some of their defense mechanisms (Fouda et al., 2019). Due to stress conditions, it causes many changes at the physiological, morphological, anatomical, biochemical and molecular levels of plants (Yılmaz et al., 2011). Abiotic stress factors are environmental factors such as cold, heat, drought, salinity, excess water, radiation, various chemicals, oxidative stress, wind and nutrient deficiency in the soil. Biotic stress factors are pathogens, insects and herbivores, including viruses, bacteria and fungi (Mahajan and Tuteja, 2005).

Effect of Biopriming on Biotic Stress

Today, in modern agriculture, advanced technologies are used to prevent crop loss and increase productivity.The use of various plant extracts, microbial products and biological agents through biopriming to protect the seed against biotic and abiotic stresses, increase plant yield and production amount (Singh et al., 2020), use less amount of chemicals, increase seed efficiency, reduce the cost of control and It is considered a sustainable approach that can be applied due to reasons such as minimal intervention to biological balance. Therefore, it offers both economic and ecological benefits by reducing the need for chemical inputs and minimizing the negative impact on the environment (Devika, 2019). Seed biopriming is an important method to control both biotic and abiotic stress factors and ensures uniform plant development under stress conditions (Prasad et al., 2016).

Seed biopriming of beneficial microorganisms is effective in reducing both soil-borne and seed-borne diseases (Rajput, 2019; Sood, 2021). This method not only increases resistance to biotic and abiotic stresses but also provides biological control before pathogen infection (Rajput, 2019). Utilized in biological control against soil pathogens, *Trichoderma* enhances nutrient uptake, releases toxins, facilitates the transfer of sugars and amino acids in plant roots, and stimulates plant growth and resilience to environmental stresses. (Zope et al., 2019; Zhang et al., 2019). In the study, biopriming method of bean seeds with *T. harzianum* reduced root rot diseases caused by *F. solani*, *R.solani* and *F. oxysporum* at pre-emergence and post-emergence stages. It has been demonstrated that the practical use of biopriming instead of chemical fungicides to control soil-borne plant pathogens that cause root rot will not pose any threat to humans, animals and the environment (El Mohamedy and Abd Alla, 2013). Additionally, seed biopriming has been found to increase disease resistance in several crops, including chickpeas, mung beans, mustard, pearl millet, and peanuts (Mondal, 2014). Similarly, in the study, it was stated that priming of *T. harzianum* in tomato increased the plant defense system against *Fusarium oxysporum* f.sp. *lycopersici* (Fol), and this was caused by increased expression of defense-related genes and increased antioxidative activities when exposed to the pathogen (Zehra et al., 2023). Phenylalanine Ammonia-Lyase

(PAL) (MacDonald and D'Cunha, 2007) and polyphenol oxidase (PPO) (Huang et al., 2018) enzymes play an important role in plant defense against various abiotic and biotic stresses. Similarly, Singh et al. (2016) reported that *T. asperellum* and Siddaiah et al. (2017) showed that in seed biopriming applications with *T. hamatum*, PAL levels, which indicate resistance to the pathogen, increased significantly compared to the control. Another study revealed that application of *Pseudomonas putida* and *Pseudomonas fluorescens* to chickpea seeds could prevent wilt disease caused by *Fusarium oxysporum* fsp *ciceris* by inhibiting it and also providing plant growth-promoting compounds (Sufyan et al.,2020). Singh et al. (2020a) revealed in their study that in maize plants bioprimed with *P. aeruginosa* MF-30, a significant increase in plant growth and antioxidant content was obtained, as well as healthier corn plants in which *Rhizoctonia solani* reduced the disease severity.

It is worth noting that the effectiveness of biopriming against plant pathogens may vary depending on the specific microorganisms utilized, the targeted pathogens, and the environmental conditions at play. Furthermore, considerations such as the timing of biopriming application and its compatibility with other agricultural practices are influential in determining its success in disease management. Ongoing researchs are actively working towards exploring and optimizing biopriming techniques for the effective and sustainable control of plant pathogens.

Effect of Biopriming on Abiotic Stress

Most abiotic environmental factors (such as salinity, drought, high and low temperatures) contain an osmotic component, leading to cellular dehydration and disrupting internal balance (homeostasis) (Mahajan and Tuteja, 2005). Environmental stressors and their adverse impact on sustainable agriculture underscore the need for innovative approaches and enhanced product varieties. This is essential to prevent the deterioration of ecological balance and meet the growing food demand of the increasing world population (Goswami et al., 2016).

For this reason, many researchers are suggesting important strategies to uphold agricultural production under stressful conditions. Approaches such as crop improvement through plant breeding, the development of transgenic plants, various chemical applications, and the use of plant growth regulators have been employed to enhance both production quantity and stress tolerance (Kerchev et al., 2020).

Biopriming is considered a significant ecological strategy utilized to boost the productivity of commonly grown varieties today and mitigate losses caused by stress. The utilization of microorganisms in the biopriming method has been described as a long-term and synergistic approach to address water scarcity in crop production (Jochum et al., 2019). The application of biopriming triggers various mechanisms that foster resistance to drought, encompassing the production of phytohormones, organic compounds, exopolysaccharides, ACC deaminase activity, and the regulation of osmolytes. Furthermore, the biopriming process provides the generation of antioxidants, activation of stress-responsive genes, and the enhancement of root development. (Khan et al., 2020; Vurukonda et al., 2016). Exopolysaccharides produced by microorganisms are biodegradable, high molecular weight polysaccharides that bring soil molecules together, maintaining water balance. In this way, they enhance soil fertility and enable plants to survive various abiotic conditions such as drought, salinity, cold, and waterlogging. Bacteria that produce exopolysaccharides, like Agrobacterium, Xanthomonas, and Enterobacter, contribute to plant health by controlling soil-borne pathogens (Pawar et al., 2013; Mahmood et al., 2016; Sanalibaba and Çakmak, 2016).

Plant growth-promoting microorganisms can reduce salt stress by increasing plant nutrient uptake or ensuring ion homeostasis, as well as by reducing oxidative stress through enhanced antioxidant activity, producing osmoprotectants, or improving photosynthesis (Pan et al., 2019). In recent studies, bacteria and fungi that stimulate plant growth and development have been used in many products to promote plant resistance to abiotic stress with the biopriming method (Forti et al., 2020). Microorganisms used for biopriming include *Bacillus polymyxa*, *Pseudomonas fluorescens*, *Gliocladium* and various *Trichoderma harzianum* species (Prasad et al., 2016).

Under salinity stress, maize seeds treated with biopriming using *Trichoderma citrinoviride* demonstrated improved phenotypic properties, biochemical characteristics, photosynthetic pigment levels, and fluorescence parameters compared to control plants (Yesilyurt et al., 2018). Similarly, biopriming of corn seeds with *Trichoderma atroviride* has been reported to increase chlorophyll and carotenoid contents in maize seedlings (Durmuş et al., 2017). Biopriming wheat with *Phanerochaete chrysosporium* has been reported to markedly alleviate the impact of salt stress, stimulate plant growth, and elevate levels of photosynthetic pigments and osmolytes (Dief et al., 2021). Similarly, under salt stress conditions, seed biopriming was carried out in rice (Dutta et al., 2023), corn (Aydınoğlu et al., 2023), tomato (Sutariati et al., 2023), wheat (Ahmadi et al., 2023), barley (Khan et al., 2023) and peas (Ghezal et al., 2016) to increase tolerance to salinity stress.

The use of plant growth-promoting rhizobacteria (PGPR) in biopriming makes a significant contribution to plant tolerance to salt stress. These rhizobacteria have been particularly emphasized for their ability to enhance plant resistance by activating the plant's defense mechanism in response to salt stress (Ha-Tran, 2021). Plants are directly or indirectly affected by abiotic stress factors such as salinity, pH, temperature, water availability, metals, and heavy metals/(loid) ions, thus creating a negative impact on plant growth and productivity (Godoy et al., 2021). Different priming techniques, including biopriming, have the capacity to alleviate symptoms of heavy metal toxicity by modulating physiological, morphological, biochemical, and molecular mechanisms in plants (Taie et al., 2019; Li et al., 2021).

In tolerating heavy metal stress, many PGPRs such as Bacillus, Pseudomonas, Methylobacterium and Streptomyces prevent heavy metal translocation to different parts of the plant (Khanna et al., 2019). Extracellular polymeric substances (EPS) containing numerous anion-binding sites, such as polysaccharides, lipopolysaccharide, glycoproteins, and soluble peptide, are released by such rhizospheric bacteria, which helps in the removal or recovery of heavy metals from the rhizosphere through biosorption (Silva et al., 2020). Seed biopriming using mycorrhiza and ectomycorrhiza not only protects the plant against heavy metal toxicity (Alguacil et al., 2011), but also provides growth promotion through secretion molecules and stimulation of changes in biochemical pathways in host plants (Tahara et al., 2005). Similarly, it supports the plant in dealing with heavy metal stress by stimulating the activities of antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and osmoprotectants (Kumar et al., 2021; Kaushik et al.,2022).

Additionally, some microorganisms produce osmolytes, reduce carbon flux, produce biofilms to cover root nodules by secreting polysaccharides, and help reduce heat stress in plants, improving the water retention ability of plant roots (Singh et al., 2019).

When some microorganisms are used in biopriming to increase tolerance to cold stress, it has been reported that plants are resistant to cold stress due to their important properties such as uptake of nutrients, Fe chelating chemicals, indole acetic acid (IAA), ACC deaminase production and bioactive molecules (Bharti et al., 2016). In conditions of cold stress, plants accumulate substantial quantities of compatible solute compounds (Ahmad et al., 2013). These compounds remain unchanged at physiological pH and exhibit non-toxic characteristics even at elevated concentrations, thereby preserving osmotic pressure. Furthermore, they contribute to the stabilization of protein and membrane structures during stress conditions (Baba et al., 2021), a factor of significance in the cell's adaptation to diverse stressors. Proline, synthesized through the glutamate and ornithine pathways, stands out as a particularly crucial compound in this process, with its production experiencing a significant boost under cold stress conditions (Ashraf and Foolad, 2007). During cold stress, free radicals increase, toxic metabolites are produced, and the development of maize plants is inhibited as membrane properties change. Biopiming with *Trichoderma* spp. regulates or eliminates free radicals. These antioxidant enzymes, capable of averting the formation of harmful compounds, enhance both the growth and yield of maize under stressful conditions by elevating physiological parameters. Hence, the application of *Trichoderma harzianum* can aid in mitigating the adverse impacts of cold stress, concurrently enhancing the physiological, morphological, and yield parameters of maize (Afrouz et al., 2023).

Out of all abiotic stresses, drought is notably one of the most crucial factors constraining crop production, and it is inevitable that this scenario will persist in the future (Passioura, 2007). Plants have evolved several mechanisms to establish resistance against drought stress. It is acknowledged that mycorrhizal fungi, crucial in regulating physiological activities, along with rhizobacteria and endophytic fungi promoting plant growth, contribute to enhancing plants' capacity to withstand abiotic stress (Ali et al.,2014; Hashem et al., 2016). In recent years, beneficial microorganisms have been providing benefits by developing resistance to drought and effectively increasing the plant's water use (Vurukonda et al., 2016). Drought is a problem that seriously affects the production of rice and other grains around the world. One of the factors hindering rice cultivation and production is water scarcity during periods of low rainfall, which affects vegetative development and productivity (Tao et al., 2006). According to a study, the biopriming application of *T. harzianum* on rice seeds was found to enhance rice development, indicating a potential positive impact on rice production, especially in drought conditions, by improving drought tolerance (Sing et al., 2023). In another study, *Bacillus pumilus* (SH-9) could increase plant tolerance to drought and exhibit different plant growth-promoting properties such as phosphate solubilization, siderophore, exopolysaccharide sucrose, and phytohormone (IAA and ABA) production. It has been stated that SH-9 plays an important role in seed biopriming, reducing stress and improving germination parameters and early seedling characteristics (Shaffique et al.,2023). It has been reported that biopriming with *T. harzianum* and *Pseudomonas fluorescens* increases the biochemical and morphological parameters of cumin under drought stress under greenhouse conditions (Piri et al., 2019). In recent studies, it has been reported that some *Bacillus* species play a role in reducing the effects of some abiotic stress factors such as heavy metals and salinity and improving plant growth (Kazerooni et al., 2021; Khan et al., 2021; Shah et al., 2021; 2020).

CONCLUSION

Biopriming is a seed treatment technique that involves the application of beneficial microorganisms, such as bacteria or fungi, to seeds before planting. This method has gained significance in modern agriculture due to its various advantages and positive impacts on plant growth, development, and biotic and abiotic stress tolerance.Various fungal or bacterial bioagents, whether acting as biofertilizers or biopesticides, may be useful as effective biopriming agents. The utility of seed biopriming extends to almost all types of cereals, pulses, vegetables, horticultural crops, and forest crops. In summary, biopriming has emerged as an important method in modern agriculture, providing sustainable solutions to enhance crop productivity, improve biotic and abiotic stress resilience, and contribute to the environmental and economic sustainability of farming practices.

REFERENCES

Abbasi, S., Safaie, N., Sadeghi, A., & Shamsbakhsh, M. (2020). Tissue-specific synergistic bio-priming of pepper by two Streptomyces species against *Phytophthora capsici*. *PloS one*, *15*(3), e0230531.

Afrouz, M., Sayyed, R.Z., Fazeli-Nasab, B., Piri, R., Almalki, W., & Fitriatin, B.N. (2023). Seed bio-priming with beneficial *Trichoderma harzianum* alleviates cold stress in maize. *PeerJ*, *11*, e15644.

Ahmad, R., Lim, C.J., & Kwon, S.Y. (2013). Glycine betaine: a versatile compound with great potential for gene pyramiding to improve crop plant performance against environmental stresses. *Plant Biotechnology Reports*, *7*, 49-57.

Ahmadi-Nouraldinvand, F., sharifi, R.S., Siadat, S.A. (2023). Reduction of Salinity Stress in Wheat through Seed Bio-Priming with Mycorrhiza and Growth-Promoting Bacteria and its Effect on Physiological Traits and Plant Antioxidant Activity with Silicon Nanoparticles Application. *Silicon* **15**, 6813–6824 https://doi.org/10.1007/s12633-023-02552-x

Alguacil, M.M., Torrecillas, E., Caravaca, F., Fernández, D.A., Azcón, R., & Roldán, A. (2011). The application of an organic amendment modifies the arbuscular mycorrhizal fungal communities colonizing native seedlings grown in a heavy-metal-polluted soil. *Soil Biology and Biochemistry*, *43*(7), 1498-1508.

Ali, S., Charles, T.C., & Glick, B.R. (2014). Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. *Plant Physiology and Biochemistry*, *80*, 160-167.

Ashraf, M.F.M.R., & Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany*, *59*(2), 206-216.

Aydinoglu, F., Kahriman, T.Y., & Balci, H. (2023). Seed bio-priming enhanced salt stress tolerance of maize (*Zea mays* L.) seedlings by regulating the antioxidant system and miRNA expression. *3 Biotech*, *13*(11), 378.

Baba Hamid, B., Sheikh, T.A., Alotaibi, S., Enshasy, H.E., Ansari, J.A., Zuan, A.T.K., & Sayyed, R.Z. (2021). Psychrotolerant *Mesorhizobium* sp. isolated from temperate and cold desert regions solubilize Potassium and produces multiple plant growth promoting metabolites. *Molecules*, *26*, 57-58.

Babu, A.N., Jogaiah, S., Ito, S.I., Nagaraj, A.K., & Tran, L.S.P. (2015). Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. *Plant Science*, *231*, 62-73.

Balasubramanian, S., Thilagar, G., Ashwin, R., & BAGYARAJ, D. (2018). Biopriming with microbial consortia on germination and growth of chilly. *J Soil Biol Ecol*, *38*, 68-74.

Begum, M.M., Sariah, M., Puteh, A.B., Abidin, M.Z., Rahman, M.A., & Siddiqui, Y. (2010). Field performance of bio-primed seeds to suppress *Colletotrichum truncatum* causing damping-off and seedling stand of soybean. *Biological Control*, *53*(1), 18-23.

Bhatt, R.M., Selvakumar, G., Upreti, K.K., & Boregowda, P.C. (2015). Effect of biopriming with Enteroacter strains on seed germination and seedling growth of tomato (*Solanum lycopersicum* L.) under osmotic stress. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 85, 63-69.

Callan, N.W., Mathre, D., & Miller, J.B. (1990). Bio-priming seed treatment for biological control of *Pythium ultimum* preemergence damping-off in sh-2 sweet corn. *Plant Disease*, *74*, 368-372.

Cantliffe, D.J. (1998). Seed germination for transplants. *Hort Technology*, *8*(4), 499-503.

Chen, K., & Arora, R. (2013). Priming memory invokes seed stress-tolerance. Environmental and experimental Botany, 94, 33-45.

Deshmukh, A.J., Jaiman, R.S., Bambharolia, R.P., & Patil, V.A. (2020). Seed biopriming-a review. *International Journal of Economic Plants*, *7*(1), 038-043.

Devika, O.S., & Rakshit, A. (2019). Economic appraisal of bio-priming mediated stress moderation in crop plants. *Economic Affairs*, *64*(3), 563-569.

Dhanya, B.A. (2014). Evaluation of Microbial Seed Priming in Relation to

Seed Germination, Plant Growth Promotion in *Morinda citrifolia* L. (Noni) (Doctoral dissertation, University of Agricultural Sciences, GKVK).

Di Vittori, L., Mazzoni, L., Battino, M., & Mezzetti, B. (2018). Pre-harvest factors influencing the quality of berries. *Scientia Horticulturae*, *233*, 310-322.

Dief, H.E.S., Hashem, E.S.A., Fawzan, S., & El-Sayed, A.S. (2021). Alleviation of salt stress in *Triticum aestivum* by biopriming with Phanerochaete chrysosporium. *Journal of Crop Science and Biotechnology*, *24*, 103-116.

Doughari, J. (2015). An overview of plant immunity. *J. Plant Pathol. Microbiol*, *6*(11), 10-4172.

Dutta, B., Datta, A., Dey, A., Ghosh, A.K., & Bandopadhyay, R. (2023). Establishment of seed biopriming in salt stress mitigation of rice plants by mangrove derived Bacillus sp. *Biocatalysis and Agricultural Biotechnology*, *48*, 102626.

El-Araby, M.M., Moustafa, S.M.A., Ismail and, A.I., & Hegazi, A.Z.A. (2006). Hormone and phenol levels during germination and osmopriming of tomato seeds, and associated variations in protein patterns and anatomical seed features. *Acta Agronomica Hungarica*, *54*(4), 441-457.

El-Mohamedy, R.S.R., & Abd Alla, M.A. (2013). Bio-priming seed treatment for biological control of soil borne fungi causing root rot of green bean (*Phaseolus vulgaris* L.). *J. Agric. Technol*, *9*(3), 589-599.

Evenari, M. (1984). Seed physiology: its history from antiquity to the beginning of the 20th century. *The Botanical Review*, *50*, 119-142.

Fallahi, H., Rezvani Moghaddam, P., Ghorbani, R., Amiri, M.B., & Fallahpour, F. (2011, April). Effects of Seed Prımıng by Biofertilizers on the Growth Characteristics of 412. Three Wheat Cultivars At The Emergence Period Under Greenhouse Condition. In 10th International Conference of the International Society for Seed Science.

Forti, C., Shankar, A., Singh, A., Balestrazzi, A., Prasad, V., & Macovei, A. (2020). Hydropriming and biopriming improve *Medicago truncatula* seed germination and upregulate DNA repair and antioxidant genes. *Genes*, *11*(3), 242.

Fouda, A., Hassan, S.E.D., Eid, A.M., & El-Din Ewais, E. (2019). The interaction between plants and bacterial endophytes under salinity stress. *Endophytes and secondary metabolites*, 1-18. https://doi.org/10.1007/978-3-319-76900-4_15-1

Ghezal, N., Rinez, I., Sbai, H., Saad, I., Farooq, M., Rinez, A., & Haouala, R. (2016). Improvement of *Pisum sativum* salt stress tolerance by bio-priming their seeds using *Typha angustifolia* leaves aqueous extract. *South African Journal of Botany*, *105*, 240-250.

Godoy, F., Olivos-Hernández, K., Stange, C., & Handford, M. (2021). Abiotic stress in crop species: improving tolerance by applying plant metabolites. *Plants*, *10*(2), 186.

Goswami, D., Thakker, J.N., & Dhandhukia, P.C. (2016). Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Cogent Food & Agriculture*, *2*(1), 1127500.

Harris, D., Joshi, A., Khan, P.A., Gothkar, P., & Sodhi, P.S. (1999). On-farm seed priming in semi-arid agriculture: development and evaluation in maize, rice and chickpea in India using participatory methods. *Experimental Agriculture*, *35*(1), 15-29.

Hashem, A., Abd_Allah, E.F., Alqarawi, A.A., Al-Huqail, A.A., Wirth, S., & Egamberdieva, D. (2016). The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of Acacia gerrardii under salt stress. *Frontiers in microbiology*, *7*, 1089.

Heydecker, W., Higgins, J., & Turner, Y.J. (1975). Invigoration of seeds. *Seed science and technology*, *3*(3/4), 881-888.

Huang, L., Jia, J., Zhao, X., Zhang, M., Huang, X., Ji, E., & Jiang, M. (2018). The ascorbate peroxidase APX1 is a direct target of a zinc finger transcription factor ZFP36 and a late embryogenesis abundant protein OsLEA5 interacts with ZFP36 to co-regulate OsAPX1 in seed germination in rice. *Biochemical and biophysical research communications*, *495*(1), 339-345.

Humphries, T., Chauhan, B.S., & Florentine, S.K. (2018). Environmental factors effecting the germination and seedling emergence of two populations of an aggressive agricultural weed; Nassella trichotoma. *PLoS One*, *13*(7), e0199491.

Imran, M., Mahmood, A., Römheld, V., & Neumann, G. (2013). Nutrient seed priming improves seedling development of maize exposed to low root zone temperatures during early growth. *European Journal of Agronomy*, *49*, 141-148.

Jochum, M.D., McWilliams, K.L., Borrego, E.J., Kolomiets, M.V., Niu, G., Pierson, E.A., & Jo, Y.K. (2019). Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. *Frontiers in microbiology*, *10*, 2106.

Kaushik, S., Sharma, P., Kaur, G., Singh, A.K., Al-Misned, F.A., Shafik, H. M., & Sirhindi, G. (2022). Seed priming with methyl jasmonate mitigates copper and cadmium toxicity by modifying biochemical attributes and antioxidants in Cajanus cajan. *Saudi Journal of Biological Sciences*, *29*(2), 721-729.

Kaymak, H.Ç., Güvenç, İ., Yarali, F., & Dönmez, M.F. (2009). The effects of bio-priming with PGPR on germination of radish (*Raphanus sativus* L.) seeds under saline conditions. *Turkish Journal of Agriculture and Forestry*, *33*(2), 173-179.

Kazerooni, E.A., Maharachchikumbura, S. S., Adhikari, A., Al-Sadi, A.M., Kang, S.M., Kim, L.R., & Lee, I.J. (2021). Rhizospheric *Bacillus amyloliquefaciens* protects *Capsicum annuum* cv. Geumsugangsan from multiple abiotic stresses via multifarious plant growth-promoting attributes. *Frontiers in plant science*, *12*, 669693.

Kerchev, P., van der Meer, T., Sujeeth, N., Verlee, A., Stevens, C.V., Van Breusegem, F., & Gechev, T. (2020). Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. *Biotechnology advances*, *40*, 107503.

Khan, I., Lubna, Asaf, S., Bilal, S., Alamri, S.S., Jan, R., & AL-Harrasi, A. (2023). Enhanced growth and stress tolerance in Barley (*Hordeum vulgare*) through biopriming with *Aspergillus niger* CSR3: a promising approach for sustainable agriculture in saline environments. *Cereal Research Communications*, 1-13.

Khan, M.A., Hamayun, M., Asaf, S., Khan, M., Yun, B.W., Kang, S.M., & Lee, I.J. (2021). Rhizospheric *Bacillus* spp. rescues plant growth under salinity stress via regulating gene expression, endogenous hormones, and antioxidant system of *Oryza sativa* L. *Frontiers in plant science*, *12*, 665590.

Khan, N., Bano, A., Ali, S., & Babar, M.A. (2020). Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. *Plant Growth Regulation*, *90*, 189-203.

Khanna, K., Jamwal, V.L., Gandhi, S.G., Ohri, P., & Bhardwaj, R. (2019). Metal resistant PGPR lowered Cd uptake and expression of metal transporter genes with improved growth and photosynthetic pigments in *Lycopersicon esculentum* under metal toxicity. *Scientific reports*, *9*(1), 5855.

Kumar, V., Hidangmayum, A., Singh, A., Sodani, R., Dadrwal, B.K., Kumar, N., & Singhal, R. K. (2021). Physiological, biochemical, and molecular mechanisms of gasotransmitter-mediated heavy metal stress tolerance in plants. *Heavy metal toxicity in plants; physiological and molecular adaptations*, *127*.

Li, Y., Liang, L., Li, W., Ashraf, U., Ma, L., Tang, X., & Mo, Z. (2021). ZnO nanoparticle-based seed priming modulates early growth and enhances physio-biochemical and metabolic profiles of fragrant rice against cadmium toxicity. *Journal of Nanobiotechnology*, *19*(1), 1-19.

MacDonald, M.J., & D’Cunha, G.B. (2007). A modern view of phenylalanine ammonia lyase. *Biochemistry and Cell Biology*, *85*(3), 273-282.

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stress: an overview. *Archives of Biochemistry and Biophysics*, 444,139-158.

Mahmood, S., Daur, I., Al-Solaimani, S.G., Ahmad, S., Madkour, M.H., Yasir, M., & Ali, Z. (2016). Plant growth promoting rhizobacteria and silicon synergistically enhance salinity tolerance of mung bean. *Frontiers in plant science*, *7*, 876.

Mavi, K. (2014). Use of extract from dry marigold (*Tagetes* spp.) flowers to prime eggplant (*Solanum melongena* L.) seeds. *Acta Scientiarum Polonorum. Hortorum Cultus*, *13*(4).

Mavi, K., & Demir, I. (2007). Controlled deterioration and accelerated aging tests predict relative seedling emergence potential of melon seed lots. *HortScience*, *42*(6), 1431-1435.

McDonald, M.B. (2000). Seed priming. *Seed technology and its biological basis*, *287*, 325.

Meyerson, F.A. (2004). Population growth and deforestation: A critical and complex relationship. *Population Reference Bureau, Washington, DC*.

Mitra, D., Mondal, R., Khoshru, B., Shadangi, S., Mohapatra, P.K.D., & Panneerselvam, P. (2021). Rhizobacteria-mediated seed bio-priming triggers the resistance and plant growth for sustainable crop production. *Current Research in Microbial Sciences*, *2*, 100071.

Mondal, S., & Bose, B. (2014). An impact of seed priming on disease resistance: A review. *Microbial diversity and biotechnology in food security*, 193-203.

Naik, M.A.N.J.U.B.A.L.A. (2015). Effect of biopriming on seed quality and management of seed borne pathogens in garden pea (*Pisum sativum* L.). *M. Sc.(Ag) thesis, Orissa University of Agriculture and Technology, 66p*.

Nile, S.H., Thiruvengadam, M., Wang, Y., Samynathan, R., Shariati, M.A., Rebezov, M., ... & Kai, G. (2022). Nano-priming as emerging seed priming technology for sustainable agriculture—recent developments and future perspectives. *Journal of nanobiotechnology*, *20*(1), 1-31.

Pan, J., Peng, F., Xue, X., You, Q., Zhang, W., Wang, T., & Huang, C. (2019). The growth promotion of two salt-tolerant plant groups with PGPR inoculation: a meta-analysis. *Sustainability*, *11*(2), 378.

Pandey, P., Irulappan, V., Bagavathiannan, M.V., & Senthil-Kumar, M. (2017). Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. *Frontiers in plant science*, *8*, 537.

Parera, C.A., & Cantliffe, D.J. (1994). Presowing seed priming. *Horticultural reviews*, *16*(16), 109-141.

Passioura, J. (2007). The drought environment: physical, biological and agricultural perspectives. *Journal of experimental botany*, *58*(2), 113-117.

Pawar, S.T., Bhosale, A.A., Gawade, T.B., & Nale, T.R. (2013). Isolation, screening and optimization of exopolysaccharide producing bacterium from saline soil. *Journal of Microbiology and Biotechnology Research*, *3*(3), 24-31.

Piri, R., Moradi, A., Balouchi, H., & Salehi, A. (2019). Improvement of cumin (*Cuminum cyminum*) seed performance under drought stress by seed coating and biopriming. *Scientia Horticulturae*, *257*, 108667.

Powell, A.A., Yule, L.J., Jing, H.C., Groot, S.P., Bino, R.J., & Pritchard, H. W. (2000). The influence of aerated hydration seed treatment on seed longevity as assessed by the viability equations. *Journal of Experimental Botany*, *51*(353), 2031-2043.

Prasad, S.R., Kamble, U.R., Sripathy, K., Bhaskar, K.U., & Singh, D. (2016). Seed bio-priming for biotic and abiotic stress management, p 211-228, Microbial inoculants in sustainable agricultural productivity.

Priya, P., Patil, V.C., & Kumar, B.A. (2016). Characterization of grain sorghum [Sorghum bicolor (L.) Moench] for root traits associated with drought tolerance. *Research in Environment and Life Sciences*, *9*(2), 163-165.

Raj, S.N., Shetty, N.P., Shetty, H.S., 2004. Seed bio-priming with *Pseudomonas fluorescens* isolates enhances growth of pearl millet plants and induces resistance against downy mil-dew. Int. J. Pest Manag. 50 (1), 41–48.

Rajendra Prasad, S., Kamble, U.R., Sripathy, K.V., Udaya Bhaskar, K., & Singh, D.P. (2016). Seed bio-priming for biotic and abiotic stress management. *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1: Research Perspectives*, 211-228.

Rajput, R.S., Singh, P., Singh, J., Ray, S., Vaishnav, A., & Singh, H.B. (2019). Seed biopriming through beneficial rhizobacteria for mitigating soil-borne and seed-borne diseases. *Plant Growth Promoting Rhizobacteria for Sustainable Stress Management: Volume 2: Rhizobacteria in Biotic Stress Management*, 201-215.

Rehman, A., & Farooq, M. (2016). Zinc seed coating improves the growth, grain yield and grain biofortification of bread wheat. *Acta Physiologiae Plantarum*, *38*, 1-10.

Roberts, D.P., Lakshman, D.K., McKenna, L.F., Emche, S.E., Maul, J.E., & Bauchan, G. (2016). Seed treatment with ethanol extract of Serratia marcescens is compatible with *Trichoderma* isolates for control of damping-off of cucumber caused by *Pythium ultimum*. *Plant Disease*, *100*(7), 1278-1287.

Sanalibaba, P., & Çakmak, G.A. (2016). Exopolysaccharides production by lactic acid bacteria. *Appl. Microbiol. open access*, *2*(1000115), 10-4172.

Shaffique, S., Imran, M., Injamum-Ul-Hoque, M., Zainurin, N., Peter, O., Alomrani, S.O., & Lee, I.J. (2023). Unraveling the new member *Bacillus pumilus* SH-9 of Bacillaceae family and its potential role in seed biopriming to mitigate drought stress in Oryza sativa. *Plant Stress*, 100318.

Shah, A.A., Aslam, S., Akbar, M., Ahmad, A., Khan, W.U., Yasin, N.A., & Ali, S. (2021). Combined effect of *Bacillus fortis* IAGS 223 and zinc oxide nanoparticles to alleviate cadmium phytotoxicity in *Cucumis melo*. *Plant Physiology and Biochemistry*, *158*, 1-12.

Shah, A.A., Bibi, F., Hussain, I., Yasin, N.A., Akram, W., Tahir, M.S., & Datta, R. (2020). Synergistic effect of *Bacillus thuringiensis* iags 199 and putrescine on alleviating cadmium-induced phytotoxicity in *Capsicum annum*. *Plants*, *9*(11), 1512.

Siddaiah, C.N., Satyanarayana, N.R., Mudili, V., Kumar Gupta, V., Gurunathan, S., Rangappa, S., & Srivastava, R.K. (2017). Elicitation of resistance and associated defense responses in *Trichoderma hamatum* induced protection against pearl millet downy mildew pathogen. *Scientific reports*, *7*(1), 43991.

Silva, R., Filgueiras, L., Santos, B., Coelho, M., Silva, M., Estrada-Bonilla, G.,& Meneses, C. (2020). *Gluconacetobacter diazotrophicus* changes the molecular mechanisms of root development in *Oryza sativa* L. growing under water stress. *International Journal of Molecular Sciences*, *21*(1), 333.

Singh, P., Singh, R., Madhu, G.S., & Singh, V.P. (2023). Seed Biopriming with *Trichoderma harzianum* for Growth Promotion and Drought Tolerance in Rice (*Oryza sativus*). *Agricultural Research*, *12*(2), 154-162.

Singh, S., Kumar, V., Dhanjal, D.S., & Singh, J. (2020). Biological control agents: diversity, ecological significances, and biotechnological applications. *Natural bioactive products in sustainable agriculture*, 31-44.

Singh, S., Singh, U.B., Malviya, D., Paul, S., Sahu, P.K., Trivedi, M., & Saxena, A. K. (2020a). Seed biopriming with microbial inoculant triggers local and systemic defense responses against *Rhizoctonia solani* causing banded leaf and sheath blight in maize (*Zea mays* L.). *International Journal of Environmental Research and Public Health*, *17*(4), 1396.

Singh, U.P., Sarma, B.K., & Singh, D.P. (2003). Effect of plant growth-promoting rhizobacteria and culture filtrate of *Sclerotium rolfsii* on phenolic and salicylic acid contents in chickpea (*Cicer arietinum*). *Current Microbiology*, *46*, 0131-0140.

Singh, V., Upadhyay, R.S., Sarma, B.K., & Singh, H.B. (2016). *Trichoderma asperellum* spore dose depended modulation of plant growth in vegetable crops. *Microbiological research*, *193*, 74-86.

Song, G.C., Choi, H.K., Kim, Y.S., Choi, J.S., & Ryu, C.M. (2017). Seed defense biopriming with bacterial cyclodipeptides triggers immunity in cucumber and pepper. *Scientific Reports*, *7*(1), 14209.

Sood, M., Kapoor, D., Kumar, V., Sheteiwy, M.S., Ramakrishnan, M., Landi, M., & Sharma, A. (2020). Trichoderma: The “secrets” of a multitalented biocontrol agent. *Plants*, *9*(6), 762.

Sowmya, K.J., Rame Gowda, R.P.S., Yogeesha, H.S., & Pallavi, H.M. (2022). Enhancement of seed quality attributes through biopriming in cucumber (*Cucumis sativus* L.).

Sufyan, M., Tahir, M.I., Haq, M.I., Hussain, S., & Saeed, M. (2020). Effect of seed bio priming with rhizobacteria against root associated pathogenic fungi in chickpea. *Pakistan Journal of Phytopathology*, *32*(1), 89-96.

Sun, H., Li, L., Wang, X., Wu, S., & Wang, X. (2011). Ascorbate–glutathione cycle of mitochondria in osmoprimed soybean cotyledons in response to imbibitional chilling injury. *Journal of Plant Physiology*, *168*(3), 226-232.

Sung, Y., Cantliffe, D.J., Nagata, R.T., & Nascimento, W.M. (2008). Structural changes in lettuce seed during germination at high temperature altered by genotype, seed maturation temperature, and seed priming. *Journal of the american society for horticultural science*, *133*(2), 300-311.

Sutariati, G.A.K., Bahrun, A., Khaeruni, A., Rahni, N.M., Rakian, T.C., Madiki, A., & Wibawa, G. N. A. (2023, September). Seed biopriming using saline soil rhizobacteria from the coast of Kolaka Regency for enhancing seed viability and vigor of tomato (*Lycopersicum esculentum* L.). In *IOP Conference Series: Earth and Environmental Science* (Vol. 1241, No. 1, p. 012017). IOP Publishing.

Tahara, K., Norisada, M., Tange, T., Yagi, H., & Kojima, K. (2005). Ectomycorrhizal association enhances Al tolerance by inducing citrate secretion in *Pinus densiflora*. *Soil Science & Plant Nutrition*, *51*(3), 397-403.

Taie, H.A., Seif El-Yazal, M.A., Ahmed, S.M., & Rady, M.M. (2019). Polyamines modulate growth, antioxidant activity, and genomic DNA in heavy metal–stressed wheat plant. *Environmental Science and Pollution Research*, *26*, 22338-22350.

Tao, H., Brueck, H., Dittert, K., Kreye, C., Lin, S., & Sattelmacher, B. (2006). Growth and yield formation of rice (*Oryza sativa* L.) in the water-saving ground cover rice production system (GCRPS). *Field Crops Research*, *95*(1), 1-12.

Taylor, A.G., Allen, P.S., Bennett, M.A., Bradford, K.J., Burris, J.S., & Misra, M. K. (1998). Seed enhancements. *Seed science research*, *8*(2), 245-256.

Thornton, J.M., & Powell, A.A. (1992). Short-term aerated hydration for the improvement of seed quality in *Brassica oleracea* L. *Seed Science Research*, *2*(1), 41-49.

Thornton, J.M., & Powell, A.A. (1995). Prolonged aerated hydration for improvement of seed quality in *Brassica oleracea* L. *Annals of Applied Biology*, *127*(1), 183-189.

Ullah, A., Farooq, M., Hussain, M., Ahmad, R., & Wakeel, A. (2019). Zinc seed coating improves emergence and seedling growth in desi and kabuli chickpea types but shows toxicity at higher concentration. *International Journal of Agriculture and Biology*, *21*(3), 553-559.

Vurukonda, S.S.K.P., Vardharajula, S., Shrivastava, M., & SkZ, A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological research*, *184*, 13-24.

Warren, J.E., & Bennett, M.A. (1997). Seed hydration using the drum priming system. *HortScience*, *32*(7), 1220-1221.

Watkins, J.T. (1998). Seed quality problems commonly encountered during vegetable and flower seed production. *Seed Technology*, 125-130.

Yeşilyurt, A.M., Pehlivan, N., Durmuş, N., & Karaoğlu, S.A. (2018). *Trichoderma citrinoviride*: a potent biopriming agent for the alleviation of salt stress in maize. *Hacettepe Journal of Biology and Chemistry*, *46*(1), 101-111.

Yılmaz, E., Tuna, A.L., & Bürün, B. (2011). Bitkilerin Tuz Stresi Etkilerine Karsı Gelistirdikleri Tolerans Stratejileri-Tolerance Strategıes Developed By Plants To The Effects Of Salt Stress. *Celal Bayar University Journal Of Science*, *7*(1), 47-66.

Zehra, A., Aamir, M., Dubey, M.K., Ansari, W. A., Meena, M., Swapnil, P., & Lee, J. (2023). Enhanced protection of tomato against Fusarium wilt through biopriming with *Trichoderma harzianum*. *Journal of King Saud University-Science*, *35*(2), 102466.

Zhang, S., Gan, Y. & Xu, B. (2019). Mechanisms of the IAA and ACC-deaminase producing strain of *Trichoderma longibrachiatum* T6 in enhancing wheat seedling tolerance to NaCl stress. *BMC Plant Biol* **19**, 22 https://doi.org/10.1186/s12870-018-1618-5

Zope, V.P., Jadhav, H.P., & Sayyed, R.Z. (2019). Neem cake carrier prolongs shelf life of biocontrol fungus *Trichoderma viridae*.

CHAPTER 5

EFFECTS OF MICROBIAL FERTILIZATION ON PARSLEY CULTIVATION

Sedanur YILMAZ[1],
Bahadır YILMAZ[1], Aslıhan Çilingir TÜTÜNCÜ[1], Abdurrahman AY[3],
Harun OZER*[1]

DOI: https://dx.doi.org/10.5281/zenodo.10450469

---

[1] Department of Horticulture, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey

[2] Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Samsun, Turkey.

[3] Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Samsun, Turkey haruno@omu.edu.tr Orchid ID 0000-0001-9106-383X

INTRODUCTION

Parsley (*Petroselinum crispum* Mill.) is a biennial aromatic plant belonging to the Apiaceae family. However, it is also a plant in the group of perennial plants because its roots can remain in the soil for many years. Although it is known that the homeland of parsley is Western Asia and South America, it is widely grown in Mediterranean countries. As in many Mediterranean countries, parsley cultivation is carried out for 12 months in our country due to its ecological diversity (Eşiyok, 2012).

Plant parts such as stems, leaves and seeds of parsley, which are consumed raw in salads and meals in our country, are a prosperous source of phytochemicals, volatile and essential fatty acids and vitamins (Kaiser et al., 2013; Meyer et al., 2006, Petropoulos et al., 2022). Thanks to these fatty acids and antioxidant capacity, it has essential contributions to treating diseases such as obesity and fatty liver and to healthy diet nutrition (Maodaa et al., 2016; Ertaş et al., 2021; Bayer, 2020).

Nitrite and nitrate accumulation threaten human health due to the intensive use of chemical fertilizers to increase yield and quality during the cultivation of parsley, which is prominent in terms of health (Cemek et al., 2007). It is reported that vegetables containing nitrite and nitrate may form carcinogenic compounds (Gajewska et al. 2009, Gruszecka and Baran 2017). For this reason, it is predicted that the health effects of parsley, a type of vegetable whose leaves are consumed, can increase exponentially with organic growing methods. In the last thirty years, microbial fertilizers have come to the fore in organic farming to reduce agricultural chemicals and increase the sustainability of production systems. Microbial fertilizers are fertilizers prepared from microorganisms that enable plants to continue their vital activities, such as growth and development in agricultural production and provide the plants with the nutrients they need. Microbial fertilizers are prepared from fungal or bacterial organisms, and natural fertilizers obtained from fungal isolates are called mycorrhiza.

For this purpose, our study investigated the effects of commercially formulated Aspergillus Oryzae fertilizer obtained from fungal isolates suitable for organic farming as an alternative to chemical fertilization on the yield and quality of parsley plants grown with organic cultivation techniques.

MATERIAL AND METHOD

The study was carried out between September and December 2022 in a greenhouse with a width of 6 m, a length of 20 m (120 m^2), and a side height of 3 m, located in the research and application land of the Faculty of Agriculture of Ondokuz Mayıs University. The study used seedlings of D'Giant Italiana parsley (*Petroselinum crispum* Mill.) variety as trial material. Planting areas have been prepared in the greenhouse where the experiment will occur. Soil samples were taken from different points at the beginning and end of the study. As a result of cultivation practices, some physical and chemical properties of the soil (pH, EC, organic matter, N, P and K), analyses of organic fertilizers (pH, EC, N, P and K) and parsley plants (dry weight, organic matter, N, P and K) analyzes determined according to Jones, (2001) and Kacar and İnal (2008) (Tables 1 and 2).

The area planted in the greenhouse was prepared by dividing the tubes into plots with a width of 1 meter, a height of 30 cm and a length of 18 meters. Drip irrigation pipes with a dripper spacing of every 20 cm were placed on the prepared tubes, suitable for double-row planting. Then, the tubes were covered with black PE mulch (1.30 m wide, 0.03 mm thick) material to combat weeds and control the high relative humidity inside the greenhouse. In the research areas, both greenhouses recorded air temperature, soil temperature and humidity values with data loggers (KT100, Kimo, France). During the cultivation period, the average air temperature in the greenhouse was determined as 17.2 °C and the average soil temperature was 18.4 °C. The lowest and highest temperature values were determined between 12-25 °C.

Ready seedlings of D'Giant Italiana parsley (*Petroselinum crispum* Mill.) variety were prepared and planted on mulch materials, with a distance of 15cm between rows and 25cm between rows. Parsley seedlings are planted in bunches and there are 8-10 sprouts in each seedling bunch.

Microbial (2g/L) fertilizer belonging to Aspergillus Oryzae (Mılıcard, MCC075 pure culture) fungi were used in the research. The chemical compound fertilizer (15-15-15) used in the research was also applied at 9.6 kg/da (N, P and K). The chemical fertilizer solution in each fertilization period was applied at approximately 1.3 ds/m EC. The first application of fertilization was made 10 days after planting. A total of 6 applications were made, the other applications being after harvest (shaping).

Additionally, a control application in which no fertilization was applied was tested. While creating the fertilization program, Ceylan et al. The criteria determined by (2005) were discussed.

Measurements and Observations Made on Parsley Plants

In the parsley grown, measurements were made on 9 plants from compound fertilizer application, biofertilizer and control applications after each cutting time.

Plant Height (cm): To determine plant height, the part of the plants from the soil surface to the tip of the crown was measured and determined as an average.

Number of Leaves (piece): The number of leaves was determined by counting the leaves attached to the main stem.

Leaf area (cm^2): Leaf area was measured with a digital planimeter (Placom Digital Planimeter, Sokkısha Planimeter Inc., Model KP-90).

Leaf chlorophyll content (CCI): Determined using Chlorophyllmeter (CCM-200, Opti-Sciences, USA).

Number of Branching: In determining the number of branching, it was calculated by counting the tillerings occurring in the plant bunches at the end of the study in each plant bunch.

Yield: Total fresh weight was weighed and determined in grams (g), and the resulting product was converted into kilograms and determined as yield per decare.

Leaf Color: Leaf color was determined by a colorimeter (Minolta, model CR–400, Tokyo, Japan) that determined leaf color in terms of CIE L*, a*, and b*. Chroma value = (a*2+b*2)1/2, and the hue angle value was determined with the formula h°= tan-1 x b*/a* (McGuire, 1992).

Amount of water-soluble dry matter (WSS): After the leaves taken from 10 plants in each repetition of each application were shredded with an electric mixer. The resulting juice was passed through cheesecloth. By taking enough leaf juice samples, readings were done on a digital refractometer (PAL^{-1}, McCormick Fruit Tech. Yakima, USA) and the values were expressed as a percentage.

Titratable acidity: To determine the TSS value, a 10 mL sample taken from the leaf juice sample was diluted with 10 mL of pure water and then titrated with 0.1 N sodium hydroxide (NaOH) until it reached pH 8.1, and citric acid was used based on the amount of NaOH spent in the titration. Expressed in (g citric acid 100 mL^{-1}).

Vitamin C: 25 g sample from parsley leaves was chopped by adding 25 ml oxalic acid (0.4%) with a blender and filtered through filter paper. The amount of vitamin C (L-ascorbic acid) in the samples taken from this filter was measured with 2,6-dichloroindophenol using the titrimetric method AOAC (1995) at a wavelength of 518 nm in a spectrophotometer and the results were given as mg vitamin C/100 g wet weight.

Statistical analysis

Experimental data were analyzed by the split-plot design with three replications with nine plants in each replication. Data were subjected to analysis of variance with SPSS (ver. 15.0, New York, USA). Means were compared with Duncan's multiple-range test ($p < 0.05$).

Results and Discussion

It was determined that the effects of different fertilizer applications (microbial and chemical) on the soil structure in the greenhouse were significant ($p < 0.05$). According to the results obtained, the highest EC (0.96 dS m^{-1}), N (0.49%), P (306.0 ppm), K (1310.0 ppm) and organic matter (7.16%) contents were obtained at the end of the study in the soil samples to which the microorganism solution was applied (Table 1). When the soil samples taken at the beginning of the study and the soil samples taken after fertilizer applications were examined, it was observed that microorganism fertilization increased the amount of P (306.0 ppm), K (1310.0 ppm) in the soil compared to chemical and control applications. At the same time, there was no difference in the amount of N (0.49%). The %N content of the soil increased with the application of microorganisms. It is reported that under normal conditions, during the decomposition of organic substances by microorganisms in the soil, microorganisms need the mineral nitrogen in the soil and the amount of nitrogen initially decreases (Durmuş and Kızılkaya, 2018). It is thought that the difference in our study is due to the high amount of organic matter in our existing soil structure.

Table 1. Soil analysis results of the growing area

	pH	EC dS/m	OM %	N %	P (ppm)	K (ppm)
Beginning	6.93	0.96 a	7.16 a	0.49 a	148.4 c	530 d
Control	6.98	0.87 ab	6.27 b	0.44 b	148.4 c	763 c
Chemical	7.09	0.78 b	7.11 a	0.44 b	255.5 b	1043 b
Microorganism	6.93	0.96 a*	7.16 a*	0.49 a*	306.0 a*	1310 a*

$^*P<0.05$

The amount of nutritional content of parsley plants was determined due to the application of chemical fertilizer and biofertilizer applications to the soil (Table 2). According to the findings, the highest plant dry weight was obtained from chemical fertilizer application. Due to microorganism fertilization, it was determined that the highest amount of N (4.09%) was obtained from parsley plants. As a result of the study, no statistical difference was observed in the organic matter, P and K contents of parsley plants. When we looked at the soil samples taken after the trial in our study, it was determined that although microorganism fertilization increased the P content in the soil, the P content in plant analyses was the same as the control application, so the plant could not benefit from the available phosphorus in the soil. In his study, Tinker (1980) stated that root infection increases to a certain P level depending on plant genotypes. However, the amount of P added after this point reduces the mycorrhizal infection of the plant. Another researcher reported that in the onion trial, the mycorrhiza increased P uptake if the available P in the soil increased up to 120 ppm. After this limit, it had no effect (Stribley, 1987). In his study on lettuce plants in 2006, Ergin reported that the phosphorus uptake of the plant decreased at a dose of 100 ppm phosphorus. In our study, the fact that the phosphorus level in the plant analysis was the same as the control is thought to be due to the P amount in the soil being above the limit values. On the other hand, researchers have demonstrated that the adherence of mycorrhiza may differ depending on plant genotypes (Smith et al., 1990; Hetrick et al., 1995; Plenchette et al., 1983).

It has been reported that mycorrhizal fungi, in addition to increasing root development in plants and providing plant nutrients and water uptake in the soil, also increase the uptake of other nutrients such as nitrogen, calcium, copper, manganese, sulfur and zinc in addition to phosphorus (Ames et al., 1983, Sieverding, 1991; Ortaş, 2002). In our study, the plant's N content increase due to microorganism application is similar to other studies. In parsley plants whose plant nutritional contents were examined, although the amount of potassium was found to be statistically insignificant, it was found to be higher in microorganism application than in chemical and control. Studies have reported that mycorrhizal applications increase the amount of K (Betlenfalvay 1982; Marschanner and Dell 1994). Sönmez (2006) reported in his study that it increased the potassium content of mycorrhiza by 5%. Our study confirms the research in this respect.

Tablo 2. Nutritional content analysis of parsley plants

	Dry Weight%	Organic matter %	N %	P %	K %
Chemical	18.6 a*	80.2	3.13 b	0.46 a	7.6 ab
Microorganism	17.8 b	82.2	4.09 a*	0.41 b	8.0 a
Control	17.6 b	81.2	3.54 b	0.41 b	7.5 ab

*P<0.05

In our study, when plant height, number of leaves, leaf area, number of branches (pieces/bunch), chlorophyll, C, Hue, SSC, titratable acidity and vitamin C parameters were examined, it was determined that there were significant differences between fertilizer applications (Table 3). The highest plant height (23.2 cm), number of leaves (8.8), leaf area (244.6 cm2), titratable acidity (0.67%) and vitamin C (43 mg 100g-1) were obtained from compound fertilizer application. The highest branching number (25.7) and H (134.4) values were obtained from microorganism application. The highest C (18.7) value was obtained in the control application. It is thought that compound fertilizers affect the plant height, number of leaves and leaf area of parsley plants because they contain more than one plant nutritional element and have a high nitrogen content. In their study, Yağmur et al., 2021 applied nitrogen, magnesium and iron to parsley plants and found that increasing doses of nitrogen fertilizer increased the vitamin C content. In their study, Saraçoğlu (1997) applied different doses of nitrogen and potassium in soilless cucumber cultivation and found that the total amount of water-soluble dry matter and titratable acidity parameters increased in parallel with the increase in nitrogen dose. Our study determined that parameters such as vitamin C, amount of water-soluble dry matter and titratable acidity were higher in the compound fertilizer application than in the mycorrhiza and control group.

Tablo 3. Effect of Compound Fertilizer and microbial fertilizer application on parsley quality criteria

	Chemical	Microorganism	Control
Plant height	23.2 a*	23.0 a	21.8 b
Leaf area	244.60 a*	172.8 b	145.6 b
Number of branches (pieces/bundle)	23.4 ab	25.7 a*	22.6 b
Yaprak klorofil içeriği	16.70	16.84	15.73
C	16.0 b	18.6 a	18.7 a*
H	125.0 b	134.4 a*	129.4 ab
SSC (%)	2.8 a*	1.6 b	2.7 a
Titratable acidity (%)	0.67 a*	0.48 b	0.54 ab
Vitamin C (mg 100g-1)	43 a*	15 c	38 b

*: $p < 0.05$. **: $p < 0.01$.

Statistically significant results were obtained for the fresh vegetative yield values of the D'Giant Italiana parsley variety grown under greenhouse conditions using biofertilizer and chemical fertilizer applications (Table 4). In the study where six different form periods were examined, it was determined that there were significant differences between the form periods. Considering the total yield values, it was determined that the highest yield was obtained from the compound fertilizer application (8.485 kg/da). It was determined that parsley plants with microorganism fertilization had the second highest yield, with a total yield of 8.395 kg/da.

Tablo 4. Yield values per decare (kg/da) according to mowing times and fertilizer applications

Reap	Chemical	Microorganism	Control
1. Reap	740 b	807 a*	610 b
2. Reap	987	1135	1024
3. Reap	1286 a*	1044 b	970 b
4. Reap	1197 b	1377 a*	792 c
5. Reap	1602 a*	1071 b	675 c
6. Reap	2673 b	2961 a*	1170 c
Average	1414 a *	1399 a	874 b
Total yield	8485 a**	8395 a	5241 b

*: $p < 0.05$. **: $p < 0.01$.

In their study, Ceylan et al., 2005 conducted 7 harvests to examine the effects of different nitrogen doses (0, 10, 20 and 30 kg da^{-1}) on the yield and some quality criteria of the parsley plant. They reported high yield values obtained at 10 kg/da N. doses. In our study, chemical fertilization with similar N content was applied and high efficiency was obtained. It has been determined that the total yield value in mycorrhizal applications is very close to chemical fertilization. As a result, when parsley plant growth and quality parameters are examined, it is thought that microorganism application can be an alternative to chemical fertilizer application.

CONCLUSION

As a result of the results obtained in our study, it was revealed that parsley plants can be grown by using mycorrhiza. The fact that the use of unconscious chemical fertilization, especially in parsley, which is an edible species, has a direct impact on human health increases the importance of fertilization in parsley cultivation. When the yield values of mycorrhiza application in parsley were examined in our study, the total yield in the chemical fertilizer application was found to be 8.495 g. In comparison, the total yield in the mycorrhiza application was 8.395 g. In addition to yield values, it is thought that mycorrhizal fertilization can be an alternative to chemical fertilization regarding plant quality parameters. It is known that the mycorrhiza used in parsley cultivation affects the plant differently depending on the genotype. For this reason, it is thought that different mycorrhiza types should be tested on different parsley varieties in future studies.

REFERENCES

Álvaro, J.E., Lao, M.T., Urrestarazu, M., Baghour, M., Abdelmajid, M. (2016). Effect of nutrient solution salinity and ionic concentration on parsley (*Petroselinum crispum Mill.*) essential oil yield and content. *Journal of Plant Nutrition*, *39*(8):1057-1062.

Ames, R.N., Reid, C.P., Porter, L.K., Camberdella, C. (1983). Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by glomus mosseae, a vesicular-arbuscular mycorrhizal fungus. *Nem Phytologist* 95(3):381-396.

Bayer, K.H. (2020)**.** Obez bireylerde maydanoz ve limon suyunun NAFLD ve obeziteye etkileri. İstanbul Medipol Üniversitesi, Sağlık Bilimleri Enstütüsü. İstanbul.

Bethlenfalvay, G.J., Brown, S.M., Pocousky, R.S. (1982). Parasitic and mutualistic asssosiations between mycorhizal fungus and soybean: Development of hast plant. *Photopatology* (72): 889-893

Cemek, M., Akkaya, L., Birdane, Y.O., Seyrek, K., Bulut, S., Konuk M. (2007). Nitrate and nitrite levels in fruity and natural mineral waters marketed in Western Turkey. *Journal of Food Composition and Analysis* 20 (3-4): 236-240.

Ceylan, Ş., Mordoğan, N., Yoldaş, F., Çakıcı, H. (2005). Azotlu gübrelemenin farklı ekim zamanlarında yetiştirilen maydanoz bitkisinde azot birikimi verim ve bazı kalite özelliklerine etkisi, Ege Üniversitesi Bilimsel Araştırma Raporu, Ödemiş, 54s.

Durmuş, M., Kızılkaya, R. (2018). Domates üretim atık ve artıklarından kompost eldesi . *Toprak Bilimi ve Bitki Besleme Dergisi* 6 (2):95-100.

Ergin, S.F. (2006). Mikorizanın marul bitkisinde bitki gelişimi ve fosfor alımı üzerine etkileri. Yüksek Lisans Tezi, Van Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, 34s, 182684.

Ertaş, B., Turan, F.B., Özbeyli, D, Yanardağ, R., Saçan, Ö., Şener, G. (2021). Protective effects of Petroselinum crispum (Parsley) extract against methotrexate-ınduced hepatotoxicity. *European Journal of Biology* 80(2):173-178.

Eşiyok, D. (2012). Kışlık ve yazlık sebze yetiştiriciliği. Ege Üniversitesi Ziraat Fakültesi Bahçe Bitkileri bölümü, Meta Basım matbaacılık hizmetleri, s. 403, İzmir.

Gajewska, M., Czajkowska, A., Bartodziejska, B. (2009). The content of nitrates (III) and (V) inselected vegetables on detail sale in °odz region. *Environ Protect Nat Resour* 40:338-95 (in Polish).

Gruszecka-Kosowska, A., Baran, A. (2017). Concentration and health risk assessment of nitrates in vegetables from conventional and organic farming. *Human and ecological risk assessment: an international journal 23*(4), 727-740.

Hetrıck, B.A.D., Kıtt, D.G., Wılson, G.T. (1986). The ınfluence of phosphorus fertilization, drought, fungal species, and non-sterile soil on mycorrhizal growth response in tall grass prairie plants. *Revue Canadienne De Botanique* 64:1199-1203.

Kaiser, A., Carle, R., Kammerer, D.R. (2013). Effects of blanching on polyphenol stability of innovative paste-like parsley (*Petroselinum crispum* (Mill.) Nym ex A. W. Hill) and marjoram (*Origanum majorana* L.) products. *Food Chemistry*. 138: 1648-1656. [CrossRef]

Maodaa, S.N., Allam, A.A., Ajarem, J., Abdel-Maksoud, M.A., Al-Basher, G.I., Wang, Z.Y. (2016). Effect of parsley (*Petroselinum crispum, Apiaceae*) juice against cadmium neurotoxicity in albino mice (Mus musculus). *Behavioral and Brain Functions* 12(1): 1-16.

Marschner, H., Dell, B. (1994). Nutrient uptake in mycorrhizal Symbiosis. *Plant soil (Netherlands*) 159: 11-25.

Meyer, H., Bolarinwa, A., Wolfram, G., Linseisen, J. (2006). Bioavailability of Apigenin from Apiin-Rich Parsley in Humans. *Annals of Nutrition Metabolism* 50:167–172.

Ortaş, İ. (2002). Do plants depend on mycorrhizae in terms of nutrient requirement? International Conference on Sustainable Land Use And Management. Çanakkale

Petropoulos, S.A. (2022). The bioactivities and chemical profile of turnip-rooted parsley germplasm. *Horticulturae* 8.7: 639.

Plenchette, C., Furlan, V., Fortın, J.A. (1983). Response of endomycorrhizal plants grown ın a calcinatyed montmorillonite clay to different levels of soluble phosphorus. Effect on growth and mycorrhizal development. *Canadian Journal of Botany* 61:1377-1383.

Saraçoğlu, A. 1997. Topraksız kültür ile yapılan sera hıyar yetiştiriciliğinde farklı dozlarda azot ve potasyum ile beslemenin bitki gelişimi, verim ve kaliteye etkileri üzerinde araştırmalar. Ege Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Yüksek Lisans Tezi. s.51, İzmir. *Sciences 6*(4), 1045-1063.

Sıeverdġng, E. (1991). Vesicular-arbuscular mycorrhizae management in tropical agrosystems. Technical Cooperation. Federal Rebpublic of Germany pp: 372.

Smıth, E.H., Walker, G., Pearson. (1990). Phsilogical interactions between symbionts in VA Mycorrhizal plants. *Annual Rewiew of Plant Physiolpgy and Plant Moleculer Biology* 39: 221-224.

Sönmez, E. (2006). Artan Dozlarda zn ve cd uygulamalarının, mikorizalı ve mikorizasız koşullarda, domates bitkisinin fide gelisimi ve N, P, K içerigine etkisi. Yüksek Lisans Tezi, Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, 35s, 199688.

Stribley, D. (1987). Mineral nutrition. In Ecophysiology of VA mycorrhizal Pants. (Ed G.R. safir) pp 59-69. CRC Press boco Raton. FL.

Şahin, H. (2015). Mikoriza, magnezyum klorür ve fosfor uygulamalarının buğdAY bitkisinin gelişimi ve beslenmesi üzerine etkisi. Yüksek Lisans Tezi, Harran üniversitesi, Fen Bilimleri Enstitüsü, 57s, 410725.

Tınker, P.B. (1980). Role of rhizosphere microorganisms in phosphorus uptake by plants. In The Role of Phosphorus in Agriculture (Eds. Khasaweneh, F.E.et.al.) ASA-CSSA-SSSA, Madison, USA.

Yağmur, B., Okur, B. (2021). Effect of nitrogen, magnesium and ıron applications on content of chlorophyll, vitamin c, nitrate and nitrite of parsley in a clay soil. *MAS Journal of Applied Sciences 6(4): 1045–1063. https://doi.org/10.52520/masjaps.161*

CHAPTER 6

BIOTIC FACTORS IN WEED BIOLOGICAL CONTROL

Derya ÖĞÜT YAVUZ[1]

DOI: https://dx.doi.org/10.5281/zenodo.10450472

---

[1] Department of Plant Protection, Faculty of Agriculture, Uşak University, Uşak, Turkiye derya.ogutyavuz@usak.edu.tr Orcid ID 0000-0001-9248-410X

INTRODUCTION

As a cornerstone of developing economies, agriculture has undertaken the crucial responsibility of ensuring food security for a healthy and prosperous life. The global agricultural area remained relatively stable from 2000 to 2020. Agricultural land encompasses 33% of the world's total land, with the remaining 67% comprising permanent meadows and pastures. Over the same period, global pesticide usage witnessed an annual increase of 1.3%. In 2020, America accounted for 51% of the total consumption, Asia 25%, Europe 18%, Africa 4%, and Oceania 3%. The rapid population growth is contributing to an escalation in food demand. The rise in food supply has given rise to concerns about food safety, and the global apprehension regarding the availability of food from the field to the table is substantial today. The American region is notable for its high chemical pesticide use per cultivated area, with intense use of chemical pesticides and fertilizers to increase agricultural production efficiency in the region. Agriculture is facing a lot of problems, including unpredictable climate change, soil contamination with pesticides and fertilizers, and a growing demand for food from a growing world population (Pouratashi and Iravani, 2012). The latest projections from the United Nations indicate that the global population is expected to reach 9.7 billion by the year 2050. It is further forecasted to peak at 10.4 billion in the 2080s and maintain that level through the year 2100. (https://www.un.org/development/desa/pd/). In agriculture, the necessity of using pesticides has arisen to sustain high yields and profitability. This scenario has ensured the ongoing production of crops across the various stages of agricultural revolutions throughout history, enabling the generation of sufficient crops to meet the needs of the expanding global population. It has also prompted numerous studies aimed at achieving higher yields. The research conducted to enhance efficiency in agriculture has yielded certain adverse effects. Despite the opening up of new agricultural areas, the negative impacts on the agroecosystem, such as decreased biodiversity and reduced productivity due to frequent ploughing, intensive fertilization, and pesticide use, have had detrimental effects on both the products and the environment. Therefore, to achieve healthy food and sustainable agricultural production, plant protection measures are becoming more and more important every day and it is necessary to effectively control the factors that cause the greatest economic loss in plant production in the short term. Biological control, a rapidly growing component in recent years, has emerged as a cornerstone of integrated pest management. It stands out as the most promising, sustainable, environmentally friendly, and highly effective among various control methods. Within this discussion, the biotic factors integral to biological weed control and outline the advantages associated with employing this method.

General problems with pesticides

Agricultural methods have led to the excessive use of pesticides and water resources in an attempt to generate more food from limited cultivable land, addressing the increasing demand of the global population. To satisfy the growing need for food, fertilizers, and pesticides are widely employed to enhance yields, with the American region ranking as the foremost user of agricultural pesticides worldwide. Global pesticide usage stood at 2 million tons in 2000, experiencing an approximately 30% increase over the next two decades. This growth equates to a compound annual rate of 1.3%, reaching 2.6 million tons by 2020. In the same year, the American region registered the highest rate of chemical pesticide application, amounting to 3.7 kg per hectare of cultivated area. Extensive research on the detrimental effects of pesticides on the environment has been conducted since the mid-20th century. Globally, efforts to address environmental issues associated with agriculture have emphasized the development of sustainable agricultural systems focused on safeguarding human health and natural resources. Worldwide, approaches to agricultural environmental problems have focused on sustainable agricultural systems that protect human health and natural resources. Agriculture stands as the largest and most vital industry, providing food for the world's population, which currently stands at roughly 8 billion people. In the present day, millions of individuals experience malnutrition and chronic hunger, even as substantial quantities of food are squandered on a daily basis. The initial stride toward guaranteeing the sustainability of the global food system and upholding food security involves evaluating agricultural practices (Struik and Kuyper, 2017). To attain sustainable agricultural production and conserve production resources, employing effective and suitable control methods is imperative.

The weed problem

It is known that agricultural production can be limited by a range of living and non-living factors. Weeds are one such factor that can contribute to decreased productivity (Chisaka, 1977; Stokstad and Grullon, 2013; Majrashi, 2022). Crop yield is greatly impacted by weeds and controlling them is an important challenge for the agriculture industry (Stokstad and Grullon, 2013). The main reason for the significant losses in crop quality and quantity, even with modern agricultural practices, is weed species- one of the most important biotic limiting factors in agriculture. About 200 of the 250,000 known species of flowering plants are regarded as important weeds with a considerable influence on agriculture (Holm et al., 1997). Worldwide, an estimated 1,800 weed species are responsible for a 31.5% reduction in crop production, which amounts to an annual economic loss of USD 32 billion (Kubiak et al., 2022). Weed-crop competition is the main factor limiting sustainable crop production (Avery, 1997). Regarding limited resources like light, water, nutrients in the soil, and space, weeds can compete successfully with crops. Weeds compete with crops for water, nutrients, and sunlight, preventing them from growing and causing significant losses in crop yield (Ali et al., 2013; Wood et al., 2015; Guglielmini et al., 2017). Crop competitive ability is influenced by genotype, crop management, and the environment. Because of characteristics like deep root systems, tolerance to cold and drought, great competitive abilities, and high nutrient usage efficiency, they can reproduce more quickly than cultivated plants. In addition, weeds can release allelopathic compounds into the soil that promote the growth of pests and plant diseases. Because of these characteristics, they can compete with crops, often resulting in lower agricultural yields and higher cultivation inputs (Trognitz et al., 2016). Weeds are commonly considered a significant barrier to the production of most crops and are estimated to cause losses of around 43% worldwide (Oerke, 2006; Neve et al., 2018).

Weeds are plants in agroecosystems that are well adapted to their environment and have been directly related to crop yield since the beginning of agriculture. Weeds are recognized as competitive, harmful, and destructive factors in croplands, causing significant losses in forestry, aquaculture, and agriculture due to their viability and adaptability (Chauhan et al., 2017; Ramesh et al., 2017). The ecological role of weeds can be viewed from various perspectives. On the one hand, weeds are perceived as pests that cause yield losses in the crop due to their competition with the crop plant (Monaco et al., 2002; Pimentel, 2005; Fontanelli et al., 2015), on the other hand, weeds are also expressed as one of the valuable agricultural ecosystem components that provide ecosystem functions and services that can support production to a certain extent, for example by protecting pollinators (Bretagnolle and Gaba, 2015). Weeds serve as a source of food and habitat for rodents, insects, and birds, as well as reduce soil erosion.

Weed management is the most widely used technique of plant protection in developed countries, both in terms of quantity and cost. Weed control methods include cultural, physical, mechanical, biological, and chemical methods. However, chemical control is often preferred due to its ease of application and ability to produce results in a short period. Herbicides, which are used to control weeds chemically, are one of the world's most widely used crop protection products. Concerns about herbicide resistance, the environmental and health threats of herbicides, and the decline of agricultural biodiversity necessitate the consideration of alternative methods for current weed management strategies that rely solely or primarily on herbicide use (Foley et al., 2011; Storkey and Neve, 2018; Adeux et al., 2019). However, an overreliance on herbicides with similar modes of action has led to the emergence of herbicide-resistant weeds. Currently, there are over 500 documented cases of herbicide-resistant weeds worldwide (Heap, 2019).

The weed flora of arable regions in Europe has undergone significant changes in recent years. Previously insignificant some weeds have become in the region's important species (Baessler and Klotz, 2006). As soon as weed control needs to adapt to these changing conditions in arable ecosystems and agronomy. Currently, there are many alternatives to controlling weeds. Biological weed control is an alternative that can be used.

Biological Control

For future agroecosystems, technologies based on ecological principles that restore ecosystem services are essential (Tilman et al., 2002). Biological control, which has rapidly increased in importance in recent years and has become the most important part of integrated management, is one of the most promising, most sustainable, environmentally friendly, and at the same time one of the most effective and applicable control methods for the protection of natural balance. It offers an alternative to relying mainly on pesticides increasing yields, which is related to damage to the environment, and risks to human health, and efficiency (Czaja et al., 2015).

Biological control is an environmentally responsible method of managing weeds. It provides innovative weed control strategies as some weeds have developed resistance to certain herbicides. Biological control agents target specific weeds. Biological control is the intentional use of a weed's natural enemies, such as insects, mites, bacteria, and fungi, to reduce its population. These control agents inhibit the growth, reproduction, and spread of the weed by feeding on it or inducing diseases in it.

Biocontrol of weeds has a long history and a success rate (Julien, 1992). Weed management by biological means has a long and effective history. In India, it was the first purposeful insect attempt to control a weed in 1836, and Hawaii had the first devoted weed management program in 1902. *Lantana camara*, a shrub-like cactus species, was introduced to the Hawaiian Islands in 1860 and spread throughout the region by birds until 1900. In 1902, natural enemies of this weed, the larvae of *Crocidosemma lantana* and *Agromyza lantanae*, were reared and released into nature, resulting in the eradication of the weed in a short time. Similar biological programs against *Lantana camara* were carried out in other regions, achieving partial success. The initial worldwide documentation of classical biological control of weeds was generated in the early 1980s (Julien 1982). Schwarzländer (2018) stated that different agents have been delivered to control weed species in various countries. In Australia, around 202 agents have been released to control 56 weed species; in Canada, 85 agents to 30 weed species; in New Zealand, 53 agents for 23 weed species; in South Africa, 103 agents for 51 weed species; and in the USA, 199 agents 74 weed species. Over 200 weed species have been targeted for biological control, and over 500 biological control agents have been intentionally released (Day and Witt, 2019). In at least one country and region, 65.7% of the target weeds for biological control have been controlled or at least to some extent (Schwarzländer et al., 2018).

Weed biocontrol is divided into two approaches: classical (inoculative or importation) and bioherbicidal (inundative or augmentative). The classical approach is the most commonly used method for managing problematic weeds in a specific region. This involves intentionally introducing specialist natural enemies from the weed's native range. It is important to note that this approach should be employed with caution and only after a thorough risk assessment. Classical biocontrol relies on introducing host-specific fungi and insects adapted to the weed for long-term control and establishment (Schwarzländer et al., 2018). Introducing a virus, herbivore, or predator to suppress the target pest is known as classical control. The environment must be conducive to the biocontrol agent's survival and population establishment for classical biocontrol to be successful (Harding and Raizada, 2015). The classical approach involves introducing organisms unique to the weed's natural habitat, such as pathogens, nematodes, and insects, into areas where the weed has become a major issue. This method aims to control the weed population by utilizing its natural enemies (Kremer, 2005). Classical biological management involves introducing a natural predator or pathogen of a pest species, aiming for its establishment in the environment to consistently reduce the population of the pest species across an entire ecosystem (Shaw et al., 2009). Classical biocontrol agents, when introduced, can cause significant damage to weeds, resulting in reductions in biomass, reproduction, and population density. This approach is widely regarded as the most cost-effective method for managing invasive weeds across various land uses (Morin, 2020).

The bioherbicide strategy, also known as inundative biological control, involves applying propagation materials such as bacterial suspensions or fungal spores in concentrations that are not typically found in nature. The goal is to eradicate the pest species within a controlled area (Johnson, 1996). To manage weeds, inundative biocontrol uses native fungi and insects that are produced in large quantities and then released (Den Breeyen et al., 2022). To achieve weed control in the year of release, the inundative technique aims to suppress a weed infestation with large quantities of a biotic agent. Timing the release of the agent to match the weed's sensitivity and formulating it to quickly target the weed host are crucial components of inundation control. The technique of bioherbicide, which applies weed pathogens in a similar way to herbicide treatments, is a development of the inundative strategy. As the inundative biological control technique can be applied in a similar way to herbicides, using inoculum in the form of liquid sprays or solid granules, it is more appropriate for crop management (Auld et al., 2003; Kremer, 2005; Harding and Raizada, 2015).

The effectiveness of biological control hinges on the ability of each agent to constrain the targeted plant, the ecological factors influencing the agent's reproduction and spread in a new environment, and the ecological dynamics of the weed determining whether the agent can significantly reduce its population. It is crucial to emphasize that the success of biological control is contingent upon meeting all three conditions (Cullen, 1995). The efficacy of biological control doesn't always result in the complete elimination of target weeds, as agents often require some surviving predator plants to fulfill their life cycle. A successful biological control strategy diminishes the potency and population of the target weed. As part of an integrated weed management plan, it is typically employed in concert with other control techniques. The selection of suitable agents has mostly been based on weed population biology, agent impact studies on the plant, and the integrated effect of herbivory and plant competitors (Gassmann, 1996). The kind of weed, its life cycle, the ecological, abiotic, and management setting in which the weed is located, and the suitable goals and effect thresholds required of a biological control program all have a significant impact on agent selection (Palmer et al., 2010).

Biological control of weeds by biotic factors

Biological control is considered the most sustainable method of weed management compared to other methods. Herbivores, natural products, plant diseases, and higher plants are typical biocontrol agents for weeds. Plant pathogens' biological control of weeds has acquired recognition as an effective, safe, and ecologically friendly weed management strategy for agroecosystems (Charudatta, 2001). The establishment, efficacy, and safety of agents are essential components of a successful weed biocontrol program (Morin, 2020). Plant diseases have been utilized in weed management since the 1960s, with some of the first efforts being the control of Rumex and Rubus spp. weed species in the United States and Chile respectively (Oehrens, 1977).

In the ecosystem, plants, pathogens, and antagonists interact. Weed invasion or successful weed management might result from an imbalance in this relationship (Den Breeyen, 2022). The target weed was consistently affected by over 20% of fungal infections released as biocontrol agents with a "heavy impact," and by another 60% with a medium or variable impact (Schwarzländer et al., 2018). Pathogens employed in biological control must have particular features such as abundance, specificity, efficacy, dissemination, and non-harm to people and animals to be successful.

Predictions of the effect prospective disease agents may have on the target weed in the new environment and data acquired early in a biocontrol program are used to determine which candidates host-specificity testing to evaluate any dangers they may represent (Sheppard, 2003). Compared to other types of pathogens, foliar fungi are typically more specific and easily spread by wind or rain splash. This is why they have been preferred in traditional biocontrol.

Bio-agents must be able to exist on or in a single species of host and cannot infect or grow on other plants. As a result, it must be host-specific. Bioagents have to throw-away from their predators and parasites. They should also be able to withstand extended or brief lack of food if the weed species for which they are designed are few. When faced with food scarcity, they must die rather than feed on other hosts. The feeding habit of the biological agent is also an important factor in weed suppression. The proliferation of bioagents should occur naturally, rapidly, and simply (Telkar et al., 2015).

Specific and nonspecific agents are categories of classical bioagent types. The first invade one or two specific weed species, while the second can attack different weeds. Specific agents: these are biological control agents that target a narrow range of host species, often specializing in one or two closely related species. They are highly adapted to a particular host and may not be effective against other, unrelated species. Nonspecific agents are biological control agents that can target a broad range of host species. They are not as host specific as specialist agents and may attack or parasitize a variety of different species. Non-specific bio agents could be fish species, snails, and mites.

There are several advantages of using microbial agents in agriculture, specifically plant pathogens. Microbial agents may be susceptible to various chemical agents, fungicides, insecticides, herbicides, and mineral liquid fertilizers. This susceptibility can be advantageous for integrated pest management strategies. Microorganisms are diverse and abundant in nature. This diversity can be harnessed for various agricultural applications, providing a range of options for pest or weed control. Microbial agents can exhibit specificity, meaning they may target particular pests, pathogens, or weeds without affecting non-target organisms. This specificity is advantageous for minimizing environmental impact. Microbial agents can be effective in controlling pests or pathogens. Their ability to colonize and interact with their target organisms can lead to successful biological control. Microbial populations can adapt and evolve over time. Microorganisms can be persistent and difficult to eradicate. This characteristic can contribute to the long-term stability of biological control strategies. Microbial agents may spread naturally, and their populations can self-regulate in response to changes in the environment or the density of their target organisms. This can lead to sustainable and self-sustaining control measures. Many microbial agents used in agriculture are harmless to humans and animals. This is important for ensuring the safety of both agricultural workers and consumers.

Fungi

Fungal pathogens are the most prominent microbial biotic agents in biocontrol studies (Hanlin 1982). These fungi can act as natural enemies of various pests, including insects, weeds, and plant pathogens. Fungal pathogens have a certain degree of host specificity, which enables targeted control of specific pest species without affecting non-target organisms. Fungi have a diverse group of species that can be used for biocontrol. Different fungal strains may target specific pests or pathogens. Fungal biocontrol agents are frequently regarded as environmentally friendly and compatible with sustainable agricultural practices. They can be incorporated into integrated pest management (IPM) strategies that reduce dependence on chemical pesticides. Suitable fungal pathogens are defined by their spores' ease of germination, dissemination, and successful overwintering strategies. Fungal pathogens with spores that can germinate rapidly are often preferred due to their ability to initiate infection promptly upon encountering their target host. Effective biocontrol depends on the fungal pathogen's capacity to locate and infect the target organisms. Fungal pathogens that can tolerate environmental stresses, such as extreme temperatures or desiccation, are more likely to persist until favorable conditions for infection arise.

The majority of weed-fungi interactions are due to diseases that cause aerial symptoms, however, these can include a soil-borne phase during their life cycle. Pathogenic agents penetrate plant tissues via stomata, specific host cells, mechanical damage sites, or when other organisms break the barrier of the epidermis. Pathogens, such as fungi, can enter plant tissues through stomatal openings. Once inside, they can infect and spread within the plant. Some pathogens have specific mechanisms for entering plant cells through interactions with specialized host cells. These interactions may involve recognition and manipulation of host cell structures for successful entry and infection. Plants may sustain mechanical injuries from a variety of sources, including herbivores, environmental factors, or human activities. Pathogens can exploit these vulnerable sites to enter plant tissues. Injured areas can provide a direct pathway for pathogens to access internal plant structures. Other organisms, such as insects or nematodes, may create wounds or openings in the plant's protective outer layer (cuticle and epidermis), which can then be exploited by pathogens to initiate infection.

Leaf staining, leaf crimp, and early leaf drop are all symptoms, as are cankers caused by various fungi (Runion et al., 2014). Weeds can be targeted by soil-borne disease fungi during seedlings' emergence and germination (Lamichhane et al. 2017). Several soil-borne fungi have the potential to cause diseases in germinating seeds and emerging seedlings. These fungi may infect seeds, roots, or other below-ground plant parts, leading to various symptoms and, in some cases, seedling death. The impact of soil-borne pathogens on weed seedlings can have implications for weed population dynamics and overall plant community composition. Damping-off attacks are caused by fungi, such as *Rhizoctonia solani* and *Fusarium* spp., are plant pathogens with a wide host spectrum that can include both weeds and crops (Ray and Vijayachandran 2013). Throughout the plant cycle, soil-borne fungal and oomycetes pathogens can attack the root systems of weeds.

Bacteria

Bacteria are excellent biocontrol possibilities in agriculture and other sectors. Bacteria often have fast growth rates in liquid culture, allowing for efficient and cost-effective mass production. This is advantageous for producing large quantities of biocontrol agents for widespread application. Bacterial biocontrol agents can be stabilized in various forms, such as frozen or dried formulations. This enhances their shelf life and facilitates storage, transportation, and application, providing flexibility and convenience for end-users. Bacteria are amenable to genetic modifications, including the selection of mutants with specific traits. This allows researchers to tailor biocontrol agents to enhance their effectiveness against target pests or pathogens. Bacteria are highly manipulable genetically, allowing scientists to introduce beneficial traits or modify existing ones. Genetic manipulation can enhance the biocontrol efficacy, environmental adaptability, and overall performance of bacterial biocontrol agents. Bacterial biocontrol agents can be applied through various methods, including spraying, soil drenching, seed coating, and incorporation into irrigation systems. This versatility in application methods increases their practicality for different agricultural systems. Bacteria have significant biocontrol potential, also the success of biocontrol strategies depends on a thorough understanding of the target organisms, the ecosystem, and the interactions between biocontrol agents and their environments.

The stability of active extracts from rhizobacterial isolates suggests that these extracts have the potential for use in field weed control (Kremer and Souissi 2001). The stability of active extracts from rhizobacterial isolates is a positive attribute that enhances the potential for these extracts to be used effectively in field weed control. It addresses practical considerations such as storage, formulation, and consistent performance, making them more viable options for sustainable and integrated weed management strategies.

Deleterious rhizobacteria (DRB), a group of microorganisms often disregarded as biological weed control agents, consist of nonparasitic bacteria (exopathogens) that inhabit plant root surfaces and have the capacity to hinder plant growth. The application of DRB for practical weed management employs an inundative strategy, aiming to introduce high bacterial numbers into the spermosphere and/or rhizosphere, leading to a swift onset of growth-inhibitory effects. This strategy mirrors the mycoherbicide approach to weed control, where agents selected for their effectiveness against specific weeds are cultured extensively in artificial media. Inocula generated from this cultivation process is then utilized for field applications during the growing season, preventing economic crop losses by effectively managing weed populations (Kremer and Kennedy 1996).

Rhizobacteria exert phytotoxic effects by producing substances that are absorbed by plants, leading to growth inhibition, reduced vigor, diminished reproductive potential, and an overall negative impact of weeds on crops. The process and substances involved in this weed suppression mechanism closely resemble plant allelopathy. Therefore, the group of rhizobacteria responsible for these activities is denoted as allelopathic bacteria (Abbas et al., 2018). The distinctive features of allelopathic bacteria present extensive opportunities for the advancement of effective biological weed control. These characteristics include extended survival in the rhizosphere, the release of secondary metabolites into the rhizosphere, and host specificity achieved through varying toxicity levels, substrate availability, and a diverse range of mechanisms or compounds produced by allelopathic bacteria (Zeller et al., 2007). The unique characteristics and mechanisms of allelopathic bacteria present possibilities for developing creative, sustainable, and eco-friendly techniques in biological weed control (Abbas et al., 2020).

Viruses

In certain instances, viruses targeting weed species have been explored as potential bioherbicides. This approach is typically contemplated for controlling invasive species within larger ecosystems rather than in narrowly managed areas. Viruses are often deemed unsuitable for inundative biological control due to their genetic variability and a lack of host specificity (Kazinczi et al., 2006).

Herbivore

Biological weed control through monophagous or oligophagous herbivores provides the chance to achieve self-sustaining, targeted weed management without ongoing costs or detrimental environmental effects (Gordon 1999). A major premise of weed biological control is that specialist herbivores released as biological control agents into the introduced range of their host plants are freed from suppression by specialist predators and parasites prevalent in their native range. The classical approach to biological weed control involves employing specialized natural enemies of the target plants to diminish their population dynamics to an economically acceptable level selectively. An essential initial phase in this method involves compiling inventories of natural enemies linked to the target weed in its native range. The specificity of a candidate biological control agent (BCA) is then thoroughly investigated to minimize adverse effects on non-target plants (Hinz et al., 2019). Examining insect attributes and fitness traits, and understanding the impact of plant resources on insect performance are steps toward enhancing the success rate of biological weed control.

CONCLUSIONS

Weeds are plant species that grow in cultivated crops, leading to reduced crop production during the cultivation period. The use of herbicides is widespread for weed control. The excessive and frequently incorrect application of herbicides has led to numerous significant adverse consequences, such as soil and groundwater contamination, the development of weed tolerance to these compounds, and harm to non-target organisms. While herbicides offer economic benefits, there is a growing concern about their adverse impact on the environment. Biological methods for weed management serve as an alternative to chemical control, addressing the environmental and health risks associated with herbicide use. The discontinuation of numerous environmentally harmful pesticides from diverse markets has underscored the necessity for new weed control methods (Charudattan, 2001). Biological weed control techniques can meet this demand.

The efficacy of biological control methods hinges on a comprehensive understanding of natural resources, ecosystems, and the environment. Recognizing the significance of preserving these elements is crucial for the adoption of sustainable agricultural practices. Although ecological advantages may result from biological control, the adoption and success of sustainable farming practices are significantly influenced by economic factors, market dynamics, and incentives. Utilizing plant pathogens as biological control agents holds promise in weed management. This approach stands as an environmentally friendly alternative to chemical methods, contributing to the principles of sustainable agriculture. The journey of studying, developing, introducing, and commercializing biological control agents is frequently a long-term undertaking. As ecological methods, herbicides, and precision agriculture advance, there is a necessity to formulate forward-looking approaches that go beyond sole reliance on chemical control. Biocontrol is acknowledged as a tool that is poised to maintain a significant role in weed management for the foreseeable future.

REFERENCES

Abbas, T., Zahir, Z.A., Naveed, M., & Kremer, R.J. (2018). Limitations of existing weed control practices necessitate development of alternative techniques based on biological approaches. *Advances in Agronomy*, *147*, 239-280.

Abbas, T., Zahir, Z.A., Naveed, M., Alwahibi, M.S., Soliman Elshikh, M., & A. El-Esawi, M. (2020). Field performance of allelopathic bacteria for biological weed control in wheat: Innovative, sustainable and eco-friendly approach for enhanced crop production. Sustainability, 12(21), 8936.

Adeux, G., Vieren, E., Carlesi, S., Bàrberi, P., Munier-Jolain, N., and Cordeau, S. (2019). Mitigating crop yield losses through weed diversity. Nature Sustainability, 2(11), 1018-1026.

Ali, A., Streibig, J.C. and Andreasen, C. Yield loss prediction models based on early estimation of weed pressure. Crop Prot. 53, 125-131 (2013).

Auld, B.A., Hetherington, S.D., & Smith, H.E. (2003). Advances in bioherbicide formulation. Weed Biology and Management, 3(2), 61-67.

Baessler C, Klotz S (2006). Effects of changes in agricultural land-use on landscape structure and arable weed vegetation over the last 50 years. Agric Ecosyst Environ 115:43–50. doi:10.1016/j.agee. 2005.12.007

Bretagnolle V., Gaba S (2015) Weeds for bees? A review. Agronomy for Sustainable Development 35, 891-909.

Charudattan, R. (2001). Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agro-ecology. BioControl, 46, 229-260.

Chauhan, B. S., Matloob, A., Mahajan, G., Aslam, F., Florentine, S. K., & Jha, P. (2017). Emerging challenges and opportunities for education and research in weed science. *Frontiers in plant science*, *8*, 1537.

Chisaka, H. (1977). Weed Damage to Crops: Yield Loss Dueto Weed Competition, Integrated Control of Weeds, ed. by J.D. Fryer and S. Matsunaka. University of Tokyo Press.1-16, Tokyo, Japan.

Cullen JM. Predicting Effectiveness: Fact and Fantasy. In: Delfosse ES, Scott RR, editors. Proc. 8th. Int Symp Biol Control Weeds. Melbourne: CSIRO; 1995. pp. 103-109

Czaja, K., Góralczyk, K., Struciński, P., Hernik, A., Korcz, W., Minorczyk, M., & Ludwicki, J. K. (2015). Biopesticides–towards increased consumer safety in the European Union. Pest management science, 71(1), 3-6.

Day, M., & Witt, A.B. (2019). Weed biological control: challenges and opportunities. *Weeds-Journal of the Asian-Pacific Weed Science Society*, *1*(2), 34-44.

De Bon H, Huat J, Parrot L, Sinzogan A, Martin T, Malezieux E, Vayssieres J-F (2014) Pesticide risks from fruit and vegetable pest management by small farmers in sub-Saharan Africa. A review. Agron Sustain Dev 34(4):723-736

Den Breeyen, A., Lange, C., & Fowler, S.V. (2022). Plant pathogens as introduced weed biological control agents: Could antagonistic fungi be important factors determining agent success or failure?. Frontiers in Fungal Biology, 3, 959753.

Foley, J. A. et al. Solutions for a cultivated planet. Nature 478, 337–342 (2011).

Fontanelli, M.; Frasconi, C.; Martelloni, L.; Pirchio, M.; Foster, R. Weed management in autumn fresh market spinach: A nonchemical alternative. HortTechnology 2015, 25, 177–184.

Gassmann, A.N.D.R.É. (1996, January). Classical biological control of weeds with insects: a case for emphasizing agent demography. In Proceedings of the IX international symposium on biological control of weeds (pp. 171-175). Rondebosch, South Africa: University of Cape Town.

Guglielmini, A., Verdú, A. and Satorre, E. (2017). Competitive ability of five common weed species in competition with soybean. Int. J. Pest Manag. 63, 30–36.

Hance, R.J. (1990). Weed control handbook: principles (No. PA 632.58 H3.). K. Holly, & B. C. P. Council (Eds.). Oxford: Blackwell Scientific Publications.

Hanlin, R.T. (1982). Identification of plant pathogenic fungi. Biological Control of Weeds with Plant Pathogens.

Harding, D.P., & Raizada, M.N. (2015). Controlling weeds with fungi, bacteria and viruses: a review. Frontiers in plant science, 6, 659.

Heap I (2019). The international survey of herbicide-resistant weeds. Available at: <http: /www.weedscience.org>.

Hinz, H.L., Winston, R.L., & Schwarzländer, M. (2019). How safe is weed biological control? A global review of direct nontarget attack. The Quarterly Review of Biology, 94(1), 1-27.

Johnson, D.R., Wyse, D.L., & Jones, K.J. (1996). Controlling weeds with phytopathogenic bacteria. Weed Technology, 10(3), 621-624.

Julien MH. (1992). Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds. Wallingford, Oxford: CAB Int. 186 pp. 3rd ed.

Kazinczi, G., Lukacs, D., Takacs, A., Horvath, J., Gaborjanyi, R., Nadasy, M., & Nadasy, E. (2006). Biological decline of Solanum nigrum due to virus infections. Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Sonderheft-, 20, 325.

Kremer, R.J. (2005). The role of bioherbicides in weed management. Biopestic. Int, 1(3), 4.

Kremer, R.J., & Kennedy, A.C. (1996). Rhizobacteria as biocontrol agents of weeds. Weed Technology, 10(3), 601-609.

Kubiak, A., Wolna-Maruwka, A., Niewiadomska, A., & Pilarska, A. A. (2022). The problem of weed infestation of agricultural plantations vs. the assumptions of the European biodiversity strategy. *Agronomy*, *12*(8), 1808.

Majrashi, A.A. Preliminary assessment of weed population in vegetable and fruit farms of Taif, Saudi Arabia. Braz. J. Biol. 2022, 82, e255816.

McFadyen REC. 1998. Biological control of weeds. Annu. Rev. Entomol. 43:369–93

Morin, L. (2020). Progress in biological control of weeds with plant pathogens. Annual review of phytopathology, 58, 201-223.

Morin, L., Reid, A. M., Sims-Chilton, N.M., Buckley, Y.M., Dhileepan, K., Hastwell, G. T., & Raghu, S. (2009). Review of approaches to evaluate the effectiveness of weed biological control agents. Biological control, 51(1), 1-15.

Neve, P., Barney, J.N., Buckley, Y., Cousens, R.D., Graham, S., Jordan, N.R., and Williams, M. (2018). Reviewing research priorities in weed ecology, evolution, and management: a horizon scan. Weed Research, 58(4), 250-258.

Oehrens, E. (1977). Biological control of the blackberry through the introduction of rust, *Phragmidium violaceum*, in Chile. Bulletin Phytosanitarie de la FAO (FAO)-Boletin Fitosanitario de la FAO (FAO).

Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31-43

Palmer, W.A., Heard, T.A., & Sheppard, A.W. (2010). A review of Australian classical biological control of weeds programs and research activities over the past 12 years. Biological Control, 52(3), 271-287.

Pimentel D, Hepperly P, Hanson J, Douds D, Seidel R (2005) Environmental, energetic, and economic comparisons of organic and conventional farming systems. BioScience 55, 573–582.

Pouratashi, M., and Iravani, H. (2012). Farmers' knowledge of integrated pest management and learning style preferences: implications for information delivery. Int. J. Pest Manage. 58, 347–353. doi: 10.1080/09670874.2012.724468.

Ramesh, K., Matloob, A., Aslam, F., Florentine, S.K., & Chauhan, B.S. (2017). Weeds in a changing climate: vulnerabilities, consequences, and implications for future weed management. *Frontiers in plant science*, *8*, 95.

Ray, P., & Vijayachandran, L.S. (2013). Evaluation of indigenous fungal pathogens from horse purslane (Trianthema portulacastrum) for their relative virulence and host range assessments to select a potential mycoherbicidal agent. Weed science, 61(4), 580-585.

Runion, G.B., Prior, S. A., Price, A.J., McElroy, J.S., & Torbert, H.A. (2014). Effects of elevated CO2 on biomass and fungi associated with two ecotypes of ragweed (*Ambrosia artemisiifolia* L.). Frontiers in plant science, 5, 500.

Schwarzländer, M., Hinz, H.L., Winston, R.L., & Day, M.D. (2018). Biological control of weeds: an analysis of introductions, rates of establishment and estimates of success, worldwide. *BioControl*, *63*, 319-331.

Shaw, R.H., Bryner, S., & Tanner, R. (2009). The life history and host range of the Japanese knotweed psyllid, *Aphalara itadori* Shinji: potentially the first classical biological weed control agent for the European Union. *Biological control*, *49*(2), 105-113.

Sheppard, A.W. (2003). Prioritising agents based on predicted efficacy: beyond the lottery approach. Improving the selection, testing and evaluation of weed biological control agents, 7, 11-21.

Stokstad, E., Grullon, G. (2013). Infographic: Pesticide planet. Science, 341, 730-731.

Storkey, J. and Neve, P. What good is weed diversity? Weed Res. 58, 239-243 (2018).

Struik, PC and Kuyper TW (2017). Sustainable intensification in agriculture: the richer shade of green. A review. for Sustainable Development 37, 39

Telkar, S.G., Gurjar, G.N., Dey, J.K., Kant, K., & Solanki, S.P.S. (2015). Biological weed control for sustainable agriculture. International Journal of Economic Plants, 2(4), 181-183.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. Nature, 418(6898), 671-677.

Trognitz, F., Hackl, E., Widhalm, S., Sessitsch, A. (2016). The role of plant-microbiome interactions in weed establishment and control. FEMS Microbiol. Ecol., 92, fiw138.

United Nations, Department of Economic and Social Affairs, Population Division (2022). World Population Prospects 2022: Ten Key Messages. July 2022. https://www.un.org/development/desa/pd/.

Wood, S.A.; Karp, D.S.; DeClerck, F.; Kremen, C.; Naeem, S.; Palm, C.A. Functional traits in agriculture: Agrobiodiversity and ecosystem services. Trends Ecol. Evol. 2015, 30, 531-539

Zeller, S.L., Brandl, H., & Schmid, B. (2007). Host-plant selectivity of rhizobacteria in a crop/weed model system.

CHAPTER 7

SALINITY STRESS IN PLANTS AND ROLE OF MICROBIAL AGENTS

Havva DİNLER[1]
Yunus KORKOM[2]
Ayşe UYSAL MORCA[3]

DOI: https://dx.doi.org/10.5281/zenodo.10450476

---

[1] Department of Plant Protection, Faculty of Agriculture, Uşak University, Uşak,Turkiye havva.dinler@usak.edu.tr Orcid ID 0000-0002-7011-5183
[2]Department of Plant Protection, Faculty of Agriculture, Aydın Adnan Menderes University, Aydın, Turkiye Orcid ID 0000-0001-5859-9026
[3]General Directorate of Agricultural Research and Policies, Ankara, Turkiye. Orcid ID 0000-0001-6871-2141

INTRODUCTION

In the twenty-first century, the world faces two major threats: climate change and global food security (Choudhury et al., 2017; Amna et al., 2021). The world population is increasing at a rate that threatens the current capacity of agricultural production, and significant improvements in agricultural production are required to meet the demands of this increase. Agricultural production needs to be increased to fulfill the nutritional demands of the expanding population, as malnutrition remains one of the major global challenges. By 2050, there will be projected 9.7 billion people on the world populations, and by 2080, there will be 10.4 billion (Anonymous, 2023). This increases the amount of crops needed to meet the nutritional demand of the population and even reduces the available agricultural land as a result of increased demand for housing areas (Watts et al., 2023).

It is estimated that the agricultural sector constitutes approximately 36 per cent of the world population as a source of food and income (Choudhury et al., 2017). Since the effect of climate change on agricultural production depends on temperature, precipitation, atmospheric CO2 and many similar factors, adverse effects on these factors cause a decrease in the amount of production (Vaishnav et al., 2019).

Therefore, in order to cope with increasing food demands, many studies have been carried out in order to increase the production decreasing due to environmental stress factors. Abiotic stress factors such as salinity, drought, cold, heat, excess water, radiation, various chemicals, oxidative stress, wind and nutrient deficiency in soil negatively affect plant health, production and yield in agricultural production. Salt stress is the most dangerous abiotic stress in arid and semi-arid regions and has a negative effect on plant growth and yield. Due to soil salinity, agricultural areas decrease by 1-2% every year, the amount of production decreases, and it has negative effects both ecologically and socio-economically (Choudhary et al., 2022).

According to FAO, more than 6% of the world's soils are affected by salinity (Parihar et al., 2015) and more than 20% of agricultural land faces salinity problems (Zhu, 2000). By 2050, it has been reported that about 50 per cent of agriculturally important land will be exposed to salt stress (Munns and Tester, 2008). Plants experience a multitude of environmental stresses, and among these, salinity is particularly deleterious, causing detrimental effects on agricultural production. Hence, salt stress is currently gaining attention as a crucial factor negatively influencing agricultural production and yield (Parihar et al., 2015).

It significantly affects fertile soils and therefore poses a serious problem in terms of both agriculture and economy (Numan et al., 2018). Soil salinity in recent years is one of the important soil destructions that seriously threatens the ecosystem. Therefore, crop production is recognized as one of the most important global problems in terms of food security and sustainable agriculture (Pistelli et al., 2023). A variety of alternative strategies, including traditional and genetic studies, have been used to improve plant growth and development and yield under conditions such as salinity and drought stress (Al-Zaban et al., 2022). Moreover, developing innovative strategies for the management and rehabilitation of salinity-affected soils is an ongoing challenge for researchers. Nonetheless, harnessing the power of beneficial microorganisms to boost both plant growth and stress resilience stands out as a highly promising strategy. The interplay between plants and various microbial species within the rhizosphere and phyllosphere induces changes in essential biological activities, coupled with the establishment of defense mechanisms to counteract diverse abiotic and biotic stresses (Oukala et al., 2021). Many physiological, biochemical and genetic properties of plants are adversely affected by stress conditions (Kumar and Verma, 2018; Fouda et al., 2019). The deterious effects of salinity on plant growth in saline soils encompass osmotic stress, ion toxicity, oxidative stress, and ethylene formation. Additionally, salinity disrupts microbiological processes, alters soil biological properties, and induces shifts in microbial communities (Choudhary et al., 2022).

Although plants have certain mechanisms to cope with stress, not every strategy is effective under all stress conditions. In this case, the microbial community, especially microorganisms living in the root and rhizosphere region of the plant, can help plants to cope with stress. A large number of microorganisms live around the roots of plants and many of them are beneficial for plants. The use of plant growth-promoting microorganisms (PGPMs) is a crucial strategy to mitigate the negative consequences of salt stress on plants, fostering plant growth through mechanisms like growth hormone secretion, increased nutrient uptake, nitrogen fixation, and disease protection (Choudhary et al., 2022).

These microorganisms are known to have a significant potential in reducing the salinity problem and its negative effects on plants. These microorganisms help plants in various ways such as maintaining osmotic balance and ion homeostasis and producing phytohormones (Choudhary et al., 2022).

Osmotic and ionic stressors that prevent plant growth are caused on by salt stress. When plants are subjected to salinity, osmotic stress occurs instantly because hypertonic conditions exist outside the cell, while ionic stress takes several days to appear because of the formation of Na+ and Cl-ions inside the cell. Osmotic stress reduces cell elongation, cell turgor pressure, and cell division in addition to altering the water balance within the cell (Vaishnav et al., 2019).

Several studies have been conducted to create stress-tolerant plant varieties using molecular and plant breeding investigations to tolerate salt stress. These techniques are known to be expensive and time-consuming, nevertheless (Gupta et al., 2022). Plants have evolved various morphological, physiological, biochemical, genetic and metabolic mechanisms against salt stress over time (Kumari et al., 2015). Nonetheless, the substantial impact of extreme salinity on crops persists, emphasizing the importance of developing and integrating diverse techniques for the cost-effective utilization of saline soils in production (Marriboina and Attipalli, 2020).

Enhancing salt tolerance in plants through the application of beneficial microbes emerges as a practical alternative strategy for managing salinity-prone soils in production areas. Some beneficial microorganisms living together with plants contribute significantly to the promotion of plant growth and salinity tolerance. These microbes regulate phytohormonal signaling, increase the interactions between soil, water, and plants, and set off a number of other processes that work connected to increase plants' resistance to salinity and drought stress (Forni et al., 2017). Plant growth-promoting bacteria (PGPB), arbuscular mycorrhizae (AMF), and different endophytes are all considered beneficial microbes. However, there remain crucial issues to be resolved in understanding both the mechanisms through which these microorganisms promote plant growth and the processes involved (Vaishnav et al., 2019).

Using microorganisms that tolerate salt to promote plant development in salinity-stressed locations is one promising strategy. Fungi and bacteria possess great potential to improve plant nutrition, crop yield, and tolerance to adverse environments. According to Zhang et al. (2019), research on the composition and activities of microbial communities in salty soils can help clarify their significant contribution to biological processes controlling nutrient cycles in saline soil. The search for beneficial symbiotic bacteria and identifying microorganisms that survive in a variety of unfavorable environmental situations (such as salinity, dryness, etc.) is therefore crucial (Ma et al., 2020).

In this chapter, information about salt stress in plants and the role of microbial agents in coping with this stress is given. Microbial agents are known to contribute significantly to the tolerance of salinity, which is one of the important stress factors in agricultural practice, and to improve the conditions.

IMPACT OF SALINITY STRESS ON PLANT

Following drought, the most serious threat to agriculture and crop production is the salinization of soil and water resources. Saline soil formation is increasing as a result of a low rainfall, high surface evaporation, weathering of local rocks, incorrect delivery method, irrigation with salty water and inadequate cultural practices. The salt stress is often seen as the poisoning of plants owing to the forming of salinity. A stress situation ensueing from a salt account high enough to led down the water likely (0.5 to 10 bar) is called NaCl stress (Yadav et al., 2019).

Salts in soil water can inhibit plant growth in 2 ways. First, they reduce the plant's ability to take up water, leading to a reduction in growth rate. This is called the osmotic or water deficit effect of salinity. Secondly, if excessive amounts of salt enter the plant through the transpiration stream, damage to cells in the transpiring leaves can occur, leading to a reduction in plant growth. This is the salinity's salt-specific or ion excess impact (Parihar et al., 2015). These and other salinity effects contribute to reduced productivity. It is forecasted that salinization will affect up to 50% of cultivated land by 2050 and worldwide economic losses from salinity stress are forecasted to be in excess of tens of billions of US dollars per year (Acharya et al., 2022). Salinity stress alters physiological, biochemical, and morphological responses in crops (Siringam et al., 2011; Yadav et al., 2019).

Salt stress inhibits plant growth and the rate of growth decrease is affected by a variety of parameters including plant species, developmental stage, and salt content (Yadav et al., 2019). Stunted growth is a survival mechanism that enables plants to against salinity stress (Munns, 2005). Salt stress has been shown to decrease the expression of critical regulatory genes involved in cell progression (e.g. cyclin and cyclin-dependent kinase), leading to a reduced number of cells in the meristem and a growth inhibition that affects the plant's ability to efficiently absorb nutrients and water (Chinnusamy and Zhu, 2003). Under salt stress, the plant cell shrinks and dehydrates; it then recovers. Cell elongation and, to a lesser extent, cell division are impaired, resulting in a slower pace of root and leaf development. Salinity stress affects lateral branch development, resulting in disparities in total growth and injury between salt-stressed and non-stressed control plants. This response is generated by changes in the cell-water connection caused by osmotic alterations except in the root (osmotic action). The osmotic action causes a degradation in the water absorption capacity in plants (Balasubramaniam et al., 2023). Although salt stress affects all developmental stages of the plant, the most affected stage is the seed production stage and therefore seed yield (Khatun & Flowers, 1995). In addition, salinity causes decreases in the number of productive flowers in the reproductive phase of plants and changes in flowering time (Munns, 2002; Çulha and Çakırlar, 2012).

In addition to changing plant growth features, salt also interferes with cell signaling, energy metabolism, and protein synthesis (Arif et al., 2020). Because of this, it limits agricultural efficiency and leads to in lower yields by required a far up metabolic consumption for crop adaptability, growth conservation, and stress reactions (Munns et al., 2015). Salinity stress suppresses seed water potential, protein content, nutrient reserve, phosphatase activity and thus has a negative effect on seed germination (Wu et al., 2019). The reduction in biomass yield and the intensity of membrane damage are caused by salt-induced osmotic stress and salt absorption rate, respectively (Volkmar & Hu, 1998). In crops, Salt slows germination by boost soluble sugar, starch, and ABA content while lowering gibberellic acid (GA3) level. Moreover, salinity stress causes a significant effect on plant yield and its components. Soil salinity reduces biomass, leaf area, yield, stem and root length (Zorb et al., 2019).

Photosynthesis is significantly affected by salt stress. A reduction in photosynthesis decreases available resources, limiting crop growth in reaction to salt (Fisarakis et al., 2001; Zhou et al., 2023). One of the reasons for the reduction in photosynthetic action is the decrease in cell permeability of CO2 as a result of dehydration of membranes. High salt concentration in the soil limits the plant's access to water, creating a high osmotic potential in the plant, but with the Osmotic stress develops in the plant as a result of a drop in water potential (Yildiz et al., 2020). Salinity inhibits increases transpiration and decreases stomatal conductivity, causing indigent gas trade and decreasing the proportion of photosynthesis (Arif et al., 2020).

The effect of salinity on photosynthesis of Medicago truncatula was associated with reduced photosystem II activity rather than limitation of stomatal conductance. Photosynthesis was reduced by the inhibition of CO2 assimilation caused by photosystem II damage (Najar et al., 2019; Yavas and Ilker, 2020). When A. thaliana was exposed to high salt concentrations, it affected the photochemistry of photosystem I and photosystem II and total leaf chlorophyll content, while Thellungiella salsuginea, a halophyte plant, was not affected (Stepien & Johnson, 2009). Salt stress affected the chloroplast structure in bean plants, and a decrease in photosynthesis rate was observed with a decrease in chlorophyll content (Ma et al., 2012). Chlorine-induced salinity caused a decrease in relative moisture content in tobacco, limiting photosynthetic capacity and consequently leading to a decrease in auxin and giberellin hormones (Wang et al., 2020). Photosynthetic electron transport is negatively affected. With the ion toxicity caused by Na+ and Cl- ions, the necessary nutrients are not available and this leads to a restriction of photosynthesis and the formation of reactive oxygen species (ROS) (Yildiz et al., 2020). ROS change photosynthetic proteins and the photosystem (Huihui et al., 2020; Balasubramaniam et al., 2023). Furthermore, short-term exposure to high concentrations of salt stress damages chloroplast ultrastructure by inducing thylakoid swelling and starch accumulation (Goussi et al., 2018; Balasubramaniam et al., 2023).

In salinity stress, the reduction in chlorophyll concentration induced by pigment photooxidation and chlorophyll degradation is an indication of oxidative stress. This leads to a decrease in chlorophyll content in plant leaves. Reduced chlorophyll content under salinity stress can trigger inactivation of photosynthesis (Ma et al., 2020). In the process of chlorophyll degradation, chlorophyll b can be converted to chlorophyll and thus chlorophyll a content can increase. Although salt stress reduces chlorophyll content, the extent of the reduction varies depending on the salt tolerance of plant species. Chlorophyll content increased in salt tolerant species and decreased in salt sensitive species (Yavas and Ilker, 2020). Furthermore, salinity causes a decrease in chlorophyll content, Chloroplast swelling, membrane loss, the emergence/development of intracellular lipid droplets, and lamellar vesiculation disruption. Low photosynthetic pigment concentration can restrict photosynthetic potential and consequently primary crop protection (Ma et al., 2020).

Salinity reduces nutrient absorption and disrupts mineral balance in the plant. A high salt content prevents absorption Zn, Fe, Mg, Ca, B, and K. Salinity reduces N, K and Zn content in leaves and P, K, Ca and Mg content in roots (Chrysargyris et al., 2019). Because salt changes the osmotic potential of the soil, plant roots have a harder time receiving mineral nutrients (Sheldon et al., 2017). High salinity increases ion toxicity and creates nutrient imbalance (Arif et al., 2020).

Plants respond to salinity in different ways. Glycophyte plants show reduced growth and total yield, while halophyte plants can easily grow and reproduce in saline conditions. Therefore, at high osmotic pressures at the root-soil interface, there is a slow effect resulting from the accumulation of Na+ and Cl^- in the leaves. This results in reduced shoot growth with reduced leaf expansion and inhibition of lateral bud formation. Increased amounts of ions like Na+ and Cl^- cause ionic toxicity in plants by disrupting ion homeostasis and making vital nutrients unavailable for plant development and metabolism. Osmotic stress and ion toxicity are responsible for the occurrence of side stresses that can impair germination, growth and development of plants (Munns, 2008, 2011; Hasanuzzaman et al., 2022).

Furthermore, increased Na+ levels lead to nutrient deficiencies by reducing the presence of additional ions like as K+, Ca2+ and Mg2+ as a result of cation conflict (Atta et al., 2019; Yildiz et al., 2020; Atta et al., 2021). During salinity, as Na+ in the growth medium increases, the plant takes up more Na+ than K+, enhancement K+ efflux out of the cell and increasing the Na/K rate (Parvin et al., 2016; Rahman et al., 2016; Atta et al., 2023).

Soil salinity is caused by ion toxicity resulting from the changing of K+ by Na+ in biochemical rebounds. K+ acts as a cofactor for many enzymes and is required in high concentrations for tRNA ribosome interaction during protein synthesis. Na+ and Cl^- cause Protein conformational alterations (Zhu 2002). Salt in the soil causes osmotic stress resulting in lack of turgidity, cell dehydration and death. Ion toxicity and osmotic stress reason metabolic instability, leading to oxidative stress (Ashraf, 2004; Fouda et al., 2019).

Salt stress reduces water potential, osmotic potential and turgor pressure in the leaf. While the toxic ion concentration increases in plant cells, the amount of K^+ and Ca^{2+} decreases; This causes ion toxicity and ROS production. The plant needs ROS level required for optimal operation; any variation in ROS concentration causes a detrimental impact on crop physiology and leads to oxidative stress (Cambridge et al., 2017; Arif et al., 2020). Water deficiency caused by salt reduces stomatal conductance, thus reducing the photosynthetic activities of plants and accelerating the accumulation of reactive oxygen species (ROS) (Hasanuzzaman et al., 2022).

Salinity causes oxidative loss in crops such as DNA injury, lipid peroxidation, enzyme inactivation, protein oxidation, hormone and nutritional imbalances, leading to the accumulation of reactive oxygen species (ROS) (Hasanuzzaman et al., 2021). ROS are mostly created in chloroplasts, mitochondria, endoplasmic reticulum, cytosol and peroxisome. Salt stress causes stomatal closure, reduces the quantity of CO_2 in leaves and stimulates photosynthetic prevention (Kamran et al., 2020; Atta et al., 2023).

Light reactions in the chloroplast result in the rapid production of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen (O_2). ROS cause oxidative damage to macromolecules like as carbohydrates, lipids, proteins, and nucleic acids leading to redox imbalance and severe oxidative stress in crops (Gill and Tuteja, 2010). Osmotic stress caused by salt stress limits the utilization of CO_2 due to stomatal closure, which increases O_2 and $^1O_2$ production in chloroplasts (Hameed et al., 2021). During salt stress, electron leakage from the electric transfer chain (ETC) causes O2 to be produced, which can then be transformed to H_2O_2 by Mn-SOD (Sharma et al 2012). Furthermore, peroxisomes are a source of ROS during salinity stress due to increased photorespiration, resulting in elevated H_2O_2 (Corpas et al., 2019; Zhou et al., 2023).

During salinity stress there is ROS induction causing oxidative stress; therefore, plants facilitate antioxidant mechanisms (Arif et al., 2020). Antioxidants are composed of enzymatic and non-enzymatic components. Superoxide dismutase (SOD), ascorbate peroxidase (APOD), glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), guaiacol peroxidase (GPOD), and catalase (KAT) are some important enzymatic components of this system. Ascorbic acid (vitamin C), glutathione, carotenoids, flavonoids and α-tocopherol (vitamin E) are non-enzymatic antioxidant compounds (Doğru and Canavar, 2020). Antioxidants reduce salinity because they are powerful ROS quenchers and scavengers. In the detoxification process in a plant cell, first, plants increase the SOD production, which results in the conversion of superoxide anion ($O2^-$) to H_2O_2, and then POX and CAT break down toxic H_2O_2 in plant cells (Zhang and Dai, 2019). The ascorbate-glutathione cycle enables the scavenging of ROS boosting the number of plant organelles ascorbate-specific peroxidase content. In halophyte plants, salinity increases the antioxidant and soluble sugar content (Singh et al., 2015; Krishnamurthy et al., 2018; Arif et al., 2020).

Salinity stress can change phytohormone production, accumulation, and distribution such as indole-3-acetic acid, abscisic acid, jasmonic acid, gibberellins, salicylic acid, and cytokinins. Thus, it promotes particular defense mechanisms (Eyidoğan et al., 2012). Abscisic acid is a key phytohormone that is susceptible to stress caused by signal detection of NaCl stress (Fahad et al., 2015; Ullah et al., 2018). Salt stress as a result of osmotic stress and water scarcity worsens abscisic acid production and distribution in vascular tissues, roots and shoots (Atta et al., 2023). Increased abscisic acid concentration triggers the influx of potassium (K) and calcium from guard cells. This leads to stomatal closure with water loss in guard cells. Abscisic acid increases the production of ROS (especially H_2O_2) in guard cells to reduce stomatal opening (stomatal closure) (Golldack et al., 2014; Mittler and Blumwald, 2015; Ma et al., 2020).

Indole-3-acetic acid has an important role in the control of plant growth. In particular, Indole-3-acetic acid is involved in cell growth, development of vascular tissue and apical dominance (Wang et al., 2001). It was reported that indole-3-acetic acid concentration in rice leaves decreased significantly under salt stress, and the decrease in indole-3-acetic acid amount caused by salt stress was partially prevented by the application of gibberalic acid (Prakash and Prathapasenan, 1990).

The biosynthesis of jasmonic acid usually occurs in chloroplasts and peroxisomes in leaves (Cheong and Choi, 2003). This hormone is also synthesized in roots. Jasmonic acid induces salinity tolerance in plants by inducing stomatal closure, scavenging ROS and promoting root development (Ma et al., 2020).

Jasmonic acid application to barley plants before salt stress positively affected growth and photosynthetic activity (Tsonev et al., 1998). It was reported that the amount of jasmonic acid increased in tolerant tomato genotypes to salt stress, while it decreased in susceptible genotypes (Pedranzani et al., 2003).

When the salt concentration exceeds the threshold salinity level, crop yields are reduced because of salt impacting reproductive structure development or nutrient reserve relocation. Salinity negatively affects crop development by lead to osmotic instability and ion toxicity. Identifying the salt stress signaling route and characterization of upstream salt stress sensors can methods for mitigating and ameliorating the harmful effects of salt stress on crops yield. While salt stress has a negative impact on plant growth and development, plants have evolved regulatory systems to cope with these harsh circumstances (Zhao et al., 2021). To comprehend the impact of salt stress on plants and the salt tolerance systems of plants, studies are needed to examine morphological, physiological, biochemical, and molecular processes of both osmotic and ion stress at the entire plant, tissue, and cellular levels. It is also necessary to determine the tolerance levels of plants to salinity and to develop tolerant plants with high nutritional values. Utilizing the potential of beneficial microorganisms, especially endophytes like as bacteria and fungi, is an alternative environmentally friendly technique for increasing plant stress resistance and better crop yields (Fouda et al., 2019). It would be a promising way to against salinity in agricultural fields and increase worldwide food breeding.

MICROBIAL ENDOPHYTES IN THE HOST PLANT

Endophytes are a widely dispersed colony of endosymbiotic bacteria that support plant growth in demanding conditions within healthy plant tissues.

They develop in the intracellular and intercellular spaces of all plant components, while they are not seen to induce infections or significant morphological problems (Ali et al., 2014).

These endophytic microbes are crucial for the growth, development, health, and diversity of plants (Fouda et al., 2019). Numerous advantageous effects of endophytic bacteria on host plants have been demonstrated, such as growth promotion, regulation of plant metabolism, and the synthesis of phytohormones that enable tolerance to biotic or abiotic stress. Because endophytic bacteria have benefits including improved disease resistance and increased plant output, they have great potential for use in agricultural applications in environments with cold, drought, salt, heavy metal stress, or disease contamination (Miliute et al., 2015; Lata et al., 2018; Fouda et al., 2019).

Endophytes play a crucial role in managing plant growth in challenging conditions through a variety of mechanisms. These microorganisms have developed both direct and indirect strategies to support host plants under unfavorable conditions. By regulating growth hormones, plant growth-promoting endophytes enhance nutrient uptake, ensuring optimal plant development in stressful and non-stressful environments alike (Ma et al., 2016; Ali et al., 2022).

The population diversity of endophytic microorganisms is directly or indirectly influenced by farming practices and various biotic/abiotic factors. Agricultural techniques, such as cultivation and irrigation, modify soil properties like disturbance, soil structure, and permeability, causing disruption to soil microbial diversity. The direct reduction in microbial diversity due to the excessive use of pesticides and fertilizers is a consequence of these practices (Tamosiune et al., 2017). Moreover, the density of endophytic populations is also shaped by microorganism-microorganism and plant-microorganism relationships (Ryan et al., 2008).

Nowadays, endophytes are widely used as biofertilizers or biological controls. Furthermore, endophytes have a wide range of uses as they can fix N_2 and CO_2, produce phytohormones, antagonistic materials and enzymes, stimulate the immune system, and compete for colonization and nutrients. They also enhance plant growth by utilizing PGPR (plant growth-promoting rhizobacteria). Endophytes settle in plant tissues through the root zone or above-ground parts, through stomata, germinating radicles, leaf tract or lateral roots. Once in the plant tissue, they colonize using certain enzymes such as pectinases, cellulases, etc. These endophytes provide various benefits to the host plant by producing antioxidants, osmolytes and Osmo protectants (Bharadwaj, 2024).

THE ROLE OF FUNGAL ENDOPHYTES IN SALINITY CONDITIONS

Plants grow under the influence of several harmful factors. These unfavorable circumstances consist of abiotic factors such as salinity, drought, cold, and heat caused by fungi, bacteria, viruses, and nematodes (Zhu, 2016; Korkom, 2023). Soil salinity induced by sodium chloride (NaCl) in agricultural production has become an issue in many nations (Gupta et al., 2020). This is a clear limitation for all plant species (Minhas and Dagar, 2016). Today, salt stress affects 20% of total farmed lands and 33% of irrigated (more than 45 M ha) agricultural regions globally (Kumar and Sharma, 2020). By 2050, more than half of agricultural fields will be salinized (Kumar and Sharma, 2020). As a result, effective measures should be developed locally and globally to ensure long-term agricultural productivity (Singh, 2022). The most promising method for increasing a plant's resistance to a range of environmental challenges is through tolerant plants as a result of plant breeding, yet this is a costly and time-consuming process (Padikasan et al., 2018). Therefore, effective, inexpensive, environmentally beneficial, and readily applied alternatives need to be investigated to reduce salinity stress.

The plant-microorganisms interaction that takes place in the rhizosphere of the plant leads to modifications in the metabolic and systemic responses of the plant under situations of salt stress (Lata et al., 2018; Jamil et al., 2022). It is also known that all plant species are in contact with at least one endophyte microorganism under natural conditions (Krings et al., 2007). Endophytic fungi have a wide range group of species that are distributed in colonizing the internal plant tissues (Azevedo et al., 2000). Plants exposed to salt experience cytotoxicity, plant mechanism disruption, reduced photosynthesis, and stunted growth and development (Isayenkov and Maathuis, 2019). Ion toxicity, oxidative stress, and osmotic shock are the first symptoms of salinity stress. These negative consequences of salinity can then expose plants to secondary pressures by reducing their capacity to uptake nutrient and H_2O (Monetti et al., 2014; Ashraf et al., 2018). In reducing these negative effects, endophytic fungi help the plant by increasing the mineral elements in the soil, and water and stimulating root development (Kohler et al., 2009; Meena et al., 2017). Endophyte fungi reduce Na^+ toxicity in the upper organs of the plant by accumulating Na^+ in root cells, vesicles, or hyphae (Khalid et al., 2018). According to several reports, fungal endophytes stimulate the plant's roots to secrete more organic compounds, which in turn influences the quantity and variety of organic acids (Zhao et al., 2014; Yang et al., 2015). For instance, fungal endophytes dissolve iron in the soil under saline conditions and make it available to the host plant (Zhao et al., 2014). This benefit provided by endophyte fungi is very important for plants under salt stress because organic acids play a role in the functions related to the tolerance level of plants against abiotic factors (Gupta et al., 2020). The different *Trichoderma* species (teleomorph *Hypocrea*) enhanced the growth of mustard (Ahmad et al., 2015), maize (Rawat et al., 2012), wheat (Rawat et al., 2011), rice (Rawat et al., 2012), *Ochradenus baccatus* (Hashem et al., 2014) plants under salt stress. They also help to minimize this stress by promoting root development (Mastouri et al., 2010) and the defense mechanism of antioxidants in plants (Ahmad et al., 2015). *T. longibrachiatum* (isolate T6) reduced the effects of salinity on wheat in an agar medium test performed under *in vitro* conditions (Zhang et al., 2016). In a similar study, it was determined that *T. harzianum* T78 successfully reduced the effect of different concentrations of salt applied to the soil (Mbarki et al., 2017). In another study, *Beauveria bassiana* isolate BeauA1 improved the root, shoot, and leaf formation of rice in salt conditions (Akter et al., 2023). *Piriformospora indica* (syn. *Serendipita indica*) is an endophyte fungus that easily colonizes plant roots and improves plant growth under salinity stress by several mechanisms (e.g. antioxidant enzymes activity, electron transfer chain, plant hormones changed) (Gill et al., 2016). This fungus has been found effective against salt stress in many plant species for instance barley (Ghaffari et al., 2019), maize (Yun et al., 2018), tomato (Ghorbani et al., 2018), mustard (Khalid et al., 2018), *Aloe barbadensis* M. (Sharma et al., 2017). One of the parameters used to determine the level of environmental stress in plants is the amount/content of chlorophyll. Salt stress significantly reduces the amount of chlorophyll in plants. Mycorrhizal fungi application was determined to decrease the negative effect of salinity stress on chlorophyll content in tomato, zucchini, and pepper plants by different researches (Colla et al., 2008; Kaya et al., 2009; Hajiboland et al., 2010). *Beauveria bassiana* isolate BeauA1 increased the chlorophyll content of rice in salinity (Akter et al., 2023). Hashem et al. (2014) have reported that *Trichoderma* inoculation enhances the chlorophyll concentration and also contributes to the biomass of the plant. Similarly, as a result of the studies carried out by Soliman et al. (2020) and Yusnawan et al. (2021), it was determined that *Trichoderma* application increased the amount of chlorophyll in the plant under salt stress. The defense mechanisms of plants under stress conditions include phytohormones, antioxidants, and various signaling pathways that stimulate the defense system (Rejeb et al., 2014). As a result of plant-microorganism interaction, the plant's defense mechanism is activated quickly and is effective in controlling salt stress (Kumar and Verma, 2018). Fungal endophytes changed the levels of phytohormones such as gibberellic acid (GA), salicylic acid (SA), indole acetic acid (IAA), and antioxidant enzymes (SOD: superoxide dismutase, APX: ascorbate peroxidase, POD: peroxidase, CAT: catalase) that play a direct act in the salt tolerance of plants are altered (Santander et al., 2020; Metwally and Soliman, 2023). Arbuscular mycorrhiza application in cucumber reduced the effect of salt stress by increasing the level of defense enzymes (Santander et al., 2020). *Trichoderma* species help plants become more tolerant of salinity by stimulating antioxidant defenses and expressing genes that tolerate salt (Brotman et al., 2013). *P. indica* showed a similar effect on sesame to activities of antioxidants (Khademian et al., 2019). GA reduces the negative effects on plants under salinity stress in two ways; i) increases antioxidant activity, and ii) promotes plant growth (Xu et al., 2018; Chauhan et al., 2019). GA-forming *Phoma herbarum* and *Penicillium* sp. (endophytic fungi) reduced salinity stress and increased the growth in cucumber and soybean (Hamayun et al., 2010; Waqas et al., 2012). Akter et al. (2023) and Zhang et al. (2019) reported that salt tolerance in plants treated with *Beauveria bassiana* (isolate BeauA1) and *T. longibrachiatum* (isolate T6) may be due to increased 1-aminocyclopropane-1-carboxylate deaminase (ACCD) activity and IAA accumulation. Endophyte inoculation normalized contents of the flavonoid and phenolic compounds in maize (Khushdil et al., 2019; Ali et al., 2022), barley (Yang et al., 2018), and groundnut (Yusnawan et al., 2021) plants under salt stress conditions. Exposure to salinity can have detrimental effects on the plant due to the production of reactive oxygen species (ROS), which include hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and superoxide anions (O_2^-) (Gengmao et al., 2014; Singh, 2022). Antioxidant metabolism is very important in land plants to ensure cell membrane integrity and control ROS in saline conditions (Huchzermeyer et al., 2022; Singh, 2022). In plant-fungi symbiosis, oxidative damage is prevented by increasing O_2^- detoxification in plants, as a result of which O_2^- is converted into H_2O_2 and other ROS, thus reducing the negative effects of salinity on the plant (Porcel et al., 2015; Khalvandi et al., 2019). The salt stress was reduced when *P. indica* was applied to tomatoes by the same mechanism (Ghorbani et al., 2018). Talaat et al. (2014) have shown that arbuscular mycorrhiza root colonization reduced salt stress in wheat by triggering the ROS defense mechanism. Proline is a molecule that initiates the function of ROS detoxification signaling pathways, and due to this property, it improves the plant's ability to tolerate abiotic stress (Hossain et al., 2014). Based on this, practices that encourage proline formation in plants are important in controlling salt stress. *P. indica* and *Azospirillum* inoculation into wheat plants caused excessive proline production in the plant (Zarea et al., 2012). Endophyte fungi were applied to rice, corn, and barrel clover plants to enhance proline synthesis under salinity stress (Khomari et al., 2018; Dief et al., 2021; Ali et al., 2022).

THE ROLE OF BACTERIAL ENDOPHYTES IN SALINITY CONDITIONS

Agricultural production is severely hampered by many abiotic stress factors such as nutrient deficiency, salinity, drought and high temperature. Bacterial endophytes are known to play a crucial role in helping plants survive these harsh conditions. Endophytic bacteria are obtained from many agricultural fields and this diversity is very important in maintaining the balance of plant physiology and maintaining the functioning of agroecosystems. In many studies, it has been reported that endophytic bacteria promote plant growth by regulating plant metabolism and phytohormone regulation and also have important effects in increasing resistance to biotic or abiotic stress factors (Fouda et al., 2019).

In saline soils, Actinobacteria, Proteobacteria, Bacteroidetes and Gemmatimonadetes, as well as Firmicutes, Acidobacteria, Nitrospirae and

Verrucomicrobia are commonly found (Rath et al., 2019). However, at high salt concentrations, the presence of Bacteroidetes and Proteobacteria was detected in small amounts, but Acidobacteria were not recorded (Tufail et al., 2021).

Endophytic bacteria, which are the most important bacteria for the development of salt tolerance in plants, are very useful. Bacterial species belonging to the genera Bacillus, Enterobacter, Burkholderia, Pseudomonas, Paraburkholderia, Pantoea, Paenibacillus and Streptomyces have been reported to significantly reduce salt stress (Ali et al., 2022).

These endophytes have developed many direct and indirect mechanisms to support the host plant in extreme environments. Studies in some plant species have reported that endophyte bacteria enhance plant growth by utilising various mechanisms under salt stress. These endophytes reduce oxidative stress and osmotic pressure, regulate amino acid and phytohormone production, and maintain nutrient balance by reducing Na+ concentration in the xylem. They also increase the surface area for greater water uptake by elongating plant roots to eliminate the effect of drought stress caused by saline conditions (Abdelaal et al., 2021; Ali et al., 2022).

There are many important scientific studies showing that there are various bacterial endophyte groups that can be effective in ameliorating salt stress in plants (Yaish et al., 2017; Lata et al., 2018; Vaishnavet et al., 2018). The maximum salt tolerance values of endophytic bacterial genera that promote plant growth under salt stress conditions are given in Figure 1 (Kushwaha et al., 2020).

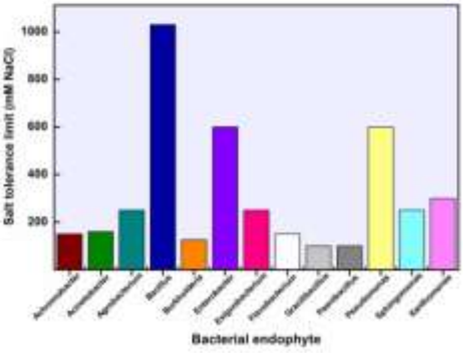


Figure 1. Salt tolerance values of some endophytic bacteria (Kushwaha et al.,2020).

Plant development has been effectively promoted by endophytes bacteria, which also lessen the negative effects of salt stress. Plants can recover from stress and develop normally when endophytic bacteria are present. They can resist salt stress by releasing osmotic pressure, eliminate harmful compounds, and boost the production of hormones including cytokines (CK) and indole acetic acid (IAA). By delaying leaf senescence and promoting osmolyte buildup, glucose metabolism, and antioxidant activity, these processes support photosynthesis (Vaishnav et al., 2019).

In addition to individual salt tolerance mechanisms of plants and bacteria, plants can increase their salinity stress tolerance by establishing symbiotic relationships with endophytic and rhizospheric bacterial communities (Cheffi et al., 2019; Hasanuzzaman et al., 2019; Sun et al., 2020). Soil salinity affects rhizospheric communities and halotolerant bacteria create a favourable environment (Stringlis et al., 2018). Endophytic bacteria stimulate plant defence mechanisms to reduce salt stress while increasing plant growth and yield (Slama et al., 2023).

Two ways that endophytes can provide plants abiotic stress tolerance are (i) by immediately activating the host's stress defense mechanisms after stress exposure, which shields plants from stress or lessens its effects (Redman et al., 1999); and (ii) via endophytes biosynthesizing anti-stress biochemicals (Schulz et al., 2002).

Antioxidants and antioxidative enzymes

Reactive oxygen species (ROS) are formed during aerobic metabolism as a result of metabolic reactions occurring in chloroplasts and mitochondria under stress conditions. While reactive oxygen species (ROS) exhibit significant benefits at low concentrations in plants, elevated levels of ROS and related redox-active compounds can lead to cell damage through oxidative stress (Farooq et al., 2019; Huang et al., 2019). Salinity stress enhances ROS production (Miller et al., 2010). Endophytic species enhance plant salt tolerance by modifying or controlling reactive oxygen species. Endophytic microbes employ mechanisms against salinity akin to those for drought tolerance. Endophytes stimulate the production of antioxidant enzymes, thereby stabilizing various free radicals and ensuring the normal functioning of cells under salt stress (Anand et al., 2023). Bacterial inoculation increased biomass and chlorophyll content in stressed plants while decreasing lipid peroxidation levels and ROS. In comparison to uninoculated plants, bacterially inoculated plants showed higher amounts of anti-oxidant enzymes, non-enzymatic anti-oxidants, SOD, POD, CAT, and GR activity, glutathione, ascorbic acid and total phenol content (Abd-Allah et al., 2018).

Phytohormone production

Beneficial microorganisms produce phytohormones, organic compounds at low concentrations that significantly enhance plant growth and yield (Verma et al., 2017). The modulation of phytohormone levels actively supports plant growth (Ali et al., 2017). Indole-3-acetic acid (IAA), a crucial phytohormone, is produced by various endophytic bacterial genera, including Bacillus, Marinobacterium, Arthrobacter, Sinorhizobium, and Pseudomonas, found in halophytic plants (Li and Jiang, 2017).

Under salt stress, IAA acts by increasing seed germination, root development and water permeability of the cell and reducing cell wall pressure (Vaishnav et al., 2019).

Endophytic bacteria produce cytokinins (CKs) to enhance plant cell divisions and enable the plant to withstand various environmental stresses, including salt stress (Akhtar et al., 2020). Inoculation of the plant with microorganisms producing phytohormones (including cytokinins) has contributed to the prevention of salt stress and plant development (Magallon and Dinneny, 2019). The role of jasmonic acid (JA) as a signaling molecule involves promoting the production of primary and secondary metabolites in plants, also fostering increased tolerance to biotic and abiotic stress (Liu et al., 2020). It was also found that inoculation of plants with bacterial agent increased JA gene expression in plants (Cassells and Rafferty-McArdle, 2011). The production of gibberellic acid (GA) by certain endophytes contributes to the stimulation of plant cell division and growth (Qin et al., 2016), seed germination, flowering and fruit development (Bhise and Dandge, 2019). In PGPB-inoculated plants under salinity stress, plant growth was promoted by producing some phytohormones, including GA. Plant growth-promoting phytohormones produced by endophytic bacteria (Haider et al., 2022) can increase the metabolism and nutrient utilization of the host plant (Phetchar and Duangpaeng, 2012; Shi et al., 2014).

Plant hormones, such as abscisic acid, cytokinins, ethylene, gibberellins, and indole-3-acetic acid (IAA), serve as crucial mediators in the intricate interaction between host plants and microorganisms. While a balanced level of ethylene is vital for regular plant growth and development, stress-induced ethylene formation can impede plant growth (Gupta et al., 2016). The production of ethylene increases, particularly under salinity stress, resulting in restricted root growth (Afridi et al., 2021). ACC deaminase-producing endophytes play a role in mitigating the detrimental effects of salinity stress on root development by converting ACC (ethylene precursor) to α-ketobutyrate and ammonia (del Carmen Orozco-Mosqueda et al., 2020). This helps to reduce ethylene concentrations, regulate plant growth and protect them from the negative effects of salt and other stress factors (Kashyap et al., 2018). In another context, ACC is utilized for nitrogen assimilation, converting into ammonia (Stringlis et al., 2018). Through ACC deaminase activity, endophytic bacteria can control how much ethylene is produced by plants. One of the characteristics of endophytes that promotes plant growth is the ACC deaminase enzyme (Glick 2014; Kumari et al., 2016).

Many plant growth-promoting bacteria (PGPB) possess the capability to produce the ACC deaminase enzyme, and these include Bacillus, Pseudomonas, Enterobacter, Acinetobacter, Arthrobacter, Corynebacterium, Serratia, Brevibacterium, Exiguobacterium, Planococcus, Micrococcus, Burkholderia, Alcaligenes, Halomonas, Zhihengliuella, Ochakrobtrum, Oceanimona, and Klebsiella (Chen et al., 2013; Sarkar et al., 2018).

Ethylene production in plants in response to stress also regulates endophytic colonisation of plant tissues. High ethylene content reduces the colonisation efficiency of bacteria with host plants (Ali et al., 2014).

Nutrient uptake regulation

The unregulated and unaware application of chemical fertilizers results in diverse environmental issues, including soil structure degradation, disturbance of soil microflora, and the accumulation of chemical residues in the food cycle. Therefore, microbial-based bio-fertilisers are important sources to replace chemical fertilisers for sustainable agriculture. Endophytic bacteria can dissolve some minerals and fix nitrogen in the atmosphere to plants (Waishnav et al., 2019).

Bacterial mechanisms to improve plant performance at high salt concentrations contribute to essential nutrient uptake and support plant biomass production. Zinc (Zn), phosphate (P) and potassium (K) are present in soil mostly in insoluble forms (Slama et al., 2019a; Slama et al., 2019b). Therefore, the solubilisation of nutrients by bacteria is crucial for plant growth. Some bacterial genera such as Bacillus, Pseudomonas, Azotobacter, Enterobacter, Providencia, Pantoea, Proteus, Serratia, Acidothiobacillus, Klebsiella, Paenibacillus provided P, Zn and K solubilization (Yadav et al., 2020; Cheffi et al., 2019; Jiang et al., 2019).

An eco-friendly way to promoting plant development and N content is by the use of N-fixing endophytic bacteria. Because endophytic bacteria can fix nitrogen in low-oxygen environments found inside plants, they are more effective at it than rhizospheric bacteria.

CONCLUSION

Its production is severely reduced in plants due to the direct impact of salt stress on functions such as photosynthesis, respiration, nutrient assimilation, hormonal imbalance, etc. With its indirect effect, it also limits production by increasing the formation of reactive oxygen species in plants under stress conditions, as well as damage to macromolecules such as proteins, lipids and nucleic acids. In many studies conducted in recent years, interest in beneficial microorganisms continues to grow. The use of microbial agents in agricultural production constitutes an increasingly developing field of research. Plant-microbial agent interaction is a very complex mechanism governed by signals, hormones, enzymes, volatile compounds, genes, and metabolites that work together to provide mutual benefit. These microbial agents are known to enhance host performance by influencing the reactions of plants under abiotic and biotic stress conditions. The effectiveness of these elements in boosting physiological performance, plant growth, root and shoot biomass, symbiotic performance, energy production, osmoregulation, Na+ accumulation, and ion homeostasis in plants, especially under salt stress conditions, is highlighted. The chapter emphasizes the essential roles played by fungi and bacteria in mitigating salt stress in plants.

REFERENCES

Abd-Allah, E.F, Alqarawi, A.A, Hashem, A., Radhakrishnan, R., Al-Huqail, A.A., Al-Otibi, F.O.L.N., Malik, J.A., Alharbi, R.I., & Egamberdieva, D. (2018) Endophytic bacterium *Bacillus subtilis* (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *J Plant Int 13*:37–44

Abdelaal, K., AlKahtani, M., Attia, K., Hafez, Y., Király, L., & Künstler, A. (2021). The role of plant growth-promoting bacteria in alleviating the adverse effects of drought on plants. *Biology*, *10*(6), 520.

Acharya, B. R., Sandhu, D., Dueñas, C., Dueñas, M., Pudussery, M., Kaundal, A., & Skaggs, T.H. (2022). Morphological, physiological, biochemical, and transcriptome studies reveal the importance of transporters and stress signaling pathways during salinity stress in Prunus. *Scientific Reports*, *12*(1), 1274.

Afridi, M.S., Van Hamme, J.D., Bundschuh, J., Sumaira, Khan, M.N., Salam, A., & Chaudhary, H.J. (2021). Biotechnological approaches in agriculture and environmental management-bacterium *Kocuria rhizophila* 14ASP as heavy metal and salt-tolerant plant growth-promoting strain. *Biologia*, *76*(10), 3091-3105.10.1007/s11756-021-00826-6

Ahmad, P., Hashem, A., Abd-Allah, E.F., Alqarawi, A.A., John, R., Egamberdieva, D., & Gucel, S. (2015). Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. *Frontiers Plant Science*, 6, 868. https://doi.org/10.3389/fpls.2015.00868

Akhtar, S.S., Mekureyaw, M.F., Pandey, C., & Roitsch, T. (2020). Role of cytokinins for interactions of plants with microbial pathogens and pest insects. *Frontiers in Plant Science*, *10*, 1777.

Akter, T., Mimma, A.A., Haque, M.A., Hossain, M.M., Ghosh, T. K., Zinan, N., & Islam, S.M.N. (2023). Seed priming with *Beauveria bassiana* improves growth and salt stress response in rice. *Environmental Experimental Botany*, 213, 105427. https://doi.org/10.1016/j.envexpbot.2023.105427

Ali, B., Hafeez, A., Javed, M. A., Afridi, M. S., Abbasi, H. A., Qayyum, A., & Selim, S. (2022). Role of endophytic bacteria in salinity stress amelioration by physiological and molecular mechanisms of defense: A comprehensive review. *South African Journal of Botany*, *151*, 33-46.

Ali, R., Gul, H., Hamayun, M., Rauf, M., Iqbal, A., Hussain, A., & Lee, I. J. (2022). Endophytic fungi controls the physicochemical status of maize crop under salt stress. *Polish Journal Environmental Studies,* 31, 561-573. https://doi.org/10.15244/pjoes/134540

Ali, S., Charles, T.C., & Glick, B.R. (2014). Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. *Plant Physiology and Biochemistry*, *80*, 160-167.

Ali, S., Charles, T.C., & Glick, B.R. (2017). Endophytic phytohormones and their role in plant growth promotion. *Functional importance of the plant microbiome: implications for agriculture, forestry and bioenergy*, 89-105.

Al-Zaban, M.I., Alhag, S.K., Dablool, A.S., Ahmed, A.E., Alghamdi, S., Ali, B., & Poczai, P. (2022). Manufactured nano-objects confer viral protection against cucurbit chlorotic yellows virus (CCYV) infecting *Nicotiana benthamiana. Microorganisms*, *10*(9), 1837.

Amna, Ali, B., Azeem, M.A., Qayyum, A., Mustafa, G., Ahmad, M.A., & Chaudhary, H. J. (2022). Bio-fabricated silver nanoparticles: A sustainable approach for augmentation of plant growth and pathogen control. In *Sustainable agriculture reviews 53: nanoparticles: a new tool to enhance stress tolerance* (pp. 345-371). Cham: Springer International Publishing.

Anand, U., Pal, T., Yadav, N., Singh, V.K., Tripathi, V., Choudhary, K.K., & Singh, A. K. (2023). Current scenario and future prospects of endophytic microbes: promising candidates for abiotic and biotic stress management for agricultural and environmental sustainability. *Microbial Ecology*, 1-32.

Anonymous, (2023). 18 Surprising Projections About the Future of Water Seametrics. https://www.seametrics.com/blog/future-water/ (accessed 13 December2023).

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A., & Hayat, S. (2020). Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry*, *156*, 64-77.

Arora, M., Saxena, P., Abdin, M. & Varma, A. (2020). Interaction between *Piriformospora indica* and *Azotobacter chroococcum* diminish the effect of salt stress in *Artemisia annua* L. by enhancing enzymatic and non-enzymatic antioxidants. *Symbiosis*, 80(6), 61–73.https://doi.org/10.1007/s13199-019-00656-w

Ashraf, M. (2004). Some important physiological selection criteria for salt tolerance in plants. *Flora-Morphology, Distribution, Functional Ecology of Plants*, *199*(5), 361-376.

Ashraf, M.A., Iqbal, M., Rasheed, R., Hussain, I., Riaz, M., & Arif, M. S. (2018). Environmental stress and secondary metabolites in plants: an overview. *Plant Metabolites and Regulation Under Environmental Stress* (pp. 153-167). Elsevier. https://doi.org/10.1016/B978-0-12-812689-9.00008-X

Atta, K., Karmakar, S., Dutta, D., Pal, A. K., & Jana, K. (2019). Comparative physiology of salinity, drought and heavy metal stress during seed germination in ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi]. *Journal of Crop and Weed*, *15*(3), 145-149. doi: 10.22271/09746315.2019.v15.i3.1251

Atta, K., Mondal, S., Gorai, S., Singh, A.P., Kumari, A., Ghosh, T., & Jespersen, D. (2023). Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. *Frontiers in Plant Science*, *14*. doi: 10.3389/fpls.2023.1241736. doi: 10.3389/fpls.2023.1241736

Atta, K., Pal, A.K., & Jana, K. (2021). Effects of salinity, drought and heavy metal stress during seed germination stage in ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi]. *Plant Physiology Reports*, *26*, 109-115. doi: 10.1007/s40502-020-00542-4

Azevedo, J.L., Maccheroni Jr, W., Pereira, J.O., & De Araújo, W. L. (2000). Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electronic journal Biotechnology*, 3(1), 15-16.

Balasubramaniam, T., Shen, G., Esmaeili, N., & Zhang, H. (2023). Plants' Response Mechanisms to Salinity Stress. *Plants*, *12*(12), 2253. https://doi.org/10.3390/plants12122253

Bharadwaj, A. (2024). Influence of endophytes on plant growth and abiotic stress. In *Plant Endophytes and Secondary Metabolites* (pp. 159-174). Academic Press.

Bharti, N., Pandey, S.S., Barnawal, D., Patel, V.K., & Kalra, A. (2016). Plant growth promoting rhizobacteria *Dietzia natronolimnaea* modulates the expression of stress responsive genes providing protection of wheat from salinity stress. *Scientific reports*, *6*(1), 34768.

Bhise, K.K., & Dandge, P.B. (2019). Mitigation of salinity stress in plants using plant growth promoting bacteria. *Symbiosis*, *79*, 191-204.

Brotman, Y., Landau, U., Cuadros-Inostroza, A., Takayuki, T., Fernie, A.R., Chet, I., Viterbo, A., & Willmitzer, L. (2013). *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. *PLoS Pathogens*, 9, e1003221 https://doi.org/10.1371/journal. ppat.1003221.

Cambridge, M.L., Zavala-Perez, A., Cawthray, G. R., Mondon, J., & Kendrick, G.A. (2017). Effects of high salinity from desalination brine on growth, photosynthesis, water relations and osmolyte concentrations of seagrass *Posidonia australis*. *Marine Pollution Bulletin*, *115*(1-2), 252-260.

Cassells, A.C., & Rafferty-McArdle, S.M. (2011). Priming of plant defences by PGPR against fungal and bacterial plant foliar pathogens. In *Bacteria in agrobiology: stress management* (pp. 1-26). Berlin, Heidelberg: Springer Berlin Heidelberg.

Chauhan, A., Amarah, B. A.A., Kumar, A., Verma, J.S., Ghramh, H.A., Khan, K.A., & Ansari, M.J. (2019). Influence of gibberellic acid and different salt concentrations on germination percentage and physiological parameters of oat cultivars. *Saudi Journal Biological Sciences,* 26 (6), 1298–1304. https://doi.org/10.1016/j.sjbs.2019.04.014.

Cheffi, M., Chenari Bouket, A., Alenezi, F.N., Luptakova, L., Belka, M., Vallat, A., & Belbahri, L. (2019). *Olea europaea* L. root endophyte *Bacillus velezensis* OEE1 counteracts oomycete and fungal harmful pathogens and harbours a large repertoire of secreted and volatile metabolites and beneficial functional genes. *Microorganisms*, *7*(9), 314.

Chen, L., Dodd, I.C., Theobald, J.C., Belimov, A.A., & Davies, W.J. (2013). The Rhizobacterium Variovorax Paradoxus 5C-2, Containing ACC Deaminase, Promotes Growth and Development of *Arabidopsis thaliana* via an Ethylene-Dependent Pathway. *J. Exp. Bot.*, 64, 1565–1573.

Cheong, J.J., & Do Choi, Y. (2003). Methyl jasmonate as a vital substance in plants. *TRENDS in Genetics*, *19*(7), 409-413. doi: 10.1016/S0168-9525(03)00138-0

Chinnusamy, V., & Zhu, J.K. (2003). Plant salt tolerance. In *Plant responses to abiotic stress* (pp. 241-270). Berlin, Heidelberg: Springer Berlin Heidelberg.

Choudhary, M., Chandra, P., Dixit, B., Nehra, V., Choudhary, U., & Choudhary, S. (2022). Plant growth-promoting microbes: Role and prospective in amelioration of salt stress. *Communications in Soil Science and Plant Analysis*, *53*(13), 1692-1711.https://doi.org/10.1080/00103624.2022.2063316

Choudhury, A., McDougall, C., Rajaratnam, S., & Park, C.M. Y. (2017). Women's empowerment in aquaculture: Two case studies from Bangladesh.

Chrysargyris, A., Papakyriakou, E., Petropoulos, S.A., & Tzortzakis, N. (2019). The combined and single effect of salinity and copper stress on growth and quality of *Mentha spicata* plants. *Journal of hazardous materials*, *368*, 584-593.

Colla, G., Rouphael, Y., Cardarelli, M., Tullio, M., Rivera, C. M., & Rea, E. (2008). Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. *Biology Fertility Soils*, 44, 501-509. https://doi.org/10.1007/s00374-007-0232-8

Corpas, F.J., Del Río, L.A., & Palma, J.M. (2019). Plant peroxisomes at the crossroad of NO and H2O2 metabolism. *Journal of integrative plant biology*, *61*(7), 803-816.

Çulha, Ş., & Çakırlar, H. (2011). Tuzluluğun bitkiler üzerine etkileri ve tuz tolerans mekanizmaları. *Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, *11*(2), 11-34.

del Carmen Orozco-Mosqueda, M., Glick, B.R., & Santoyo, G. (2020). ACC deaminase in plant growth-promoting bacteria (PGPB): An efficient mechanism to counter salt stress in crops. *Microbiological Research*, *235*, 126439.

Dief, H.E.S., Hashem, E.S.A., Fawzan, S., & El-Sayed, A.S. (2021). Alleviation of salt stress in *Triticum aestivum* by biopriming with *Phanerochaete chrysosporium*. *Journal Crop Science Biotechnology*, 24, 103-116. https://doi.org/10.1007/s12892-020-00064-3

Dodd, I.C., & Perez-Alfocea, F. (2012). Microbial amelioration of crop salinity stress. *Journal of experimental botany*, *63*(9), 3415-3428.

Doğru, A., & Canavar, S. (2020). Bitkilerde tuz toleransının fizyolojik ve biyokimyasal bileşenleri. *Academic Platform-Journal of Engineering and Science*, *8*(1), 155-174.

Eyidogan, F., Oz, M. T., Yucel, M., & Oktem, H. A. (2012). Signal transduction of phytohormones under abiotic stresses. *Phytohormones and abiotic stress tolerance in plants*, 1-48.

Fahad, S., Hussain, S., Matloob, A., Khan, F. A., Khaliq, A., Saud, S., & Huang, J. (2015). Phytohormones and plant responses to salinity stress: a review. *Plant growth regulation*, *75*, 391-404. doi: 10.1007/s10725-014-0013-y

Fisarakis, I., Chartzoulakis, K., & Stavrakas, D. (2001). Response of Sultana vines (*V. vinifera* L.) on six rootstocks to NaCl salinity exposure and recovery. *Agricultural water management*, *51*(1), 13-27.

Forni, C., Duca, D., & Glick, B.R. (2017). Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. *Plant and Soil*, *410*, 335-356.

Fouda, A., Hassan, S.E.D., Eid, A.M., & El-Din Ewais, E. (2019). The interaction between plants and bacterial endophytes under salinity stress. *Endophytes and secondary metabolites*, 1-18. https://doi.org/10.1007/978-3-319-76900-4_15-1

García-Orenes, F., Morugán-Coronado, A., Zornoza, R., & Scow, K. (2013). Changes in soil microbial community structure influenced by agricultural management practices in a Mediterranean agro-ecosystem. *PloS one*, *8*(11), e80522.

Gengmao, Z., Yu, H., Xing, S., Shihui, L., Quanmei, S., & Changhai, W. (2015). Salinity stress increases secondary metabolites and enzyme activity in safflower. *Industrial Crops Products*, 64, 175-181. https://doi.org/10.1016/j.indcrop.2014.10.058

Ghaffari, M.R., Mirzaei, M., Ghabooli, M., Khatabi, B., Wu, Y., Zabet-Moghaddam, M., & Salekdeh, G.H. (2019). Root endophytic fungus *Piriformospora indica* improves drought stress adaptation in barley by metabolic and proteomic reprogramming. *Environmental Experimental Botany*, 157, 197-210. https://doi.org/10.1016/j.envexpbot.2018.10.002

Ghorbani, A., Razavi, S.M., Omran, V.G., & Pirdashti, H. (2018). *Piriformospora indica* alleviates salinity by boosting redox poise and antioxidative potential of tomato. *Russian Journal Plant Physiology*, 65, 898-907. https://doi.org/10.1134/S1021443718060079

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, *48*(12), 909-930.

Gill, S. S., Gill, R., Trivedi, D.K., Anjum, N.A., Sharma, K. K., Ansari, M.W., & Tuteja, N. (2016). *Piriformospora indica*: potential and significance in plant stress tolerance. *Frontiers Microbiology*, 7, 332. https://doi.org/10.3389/fmicb.2016.00332

Glick, B.R. (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39

Golldack, D., Li, C., Mohan, H., & Probst, N. (2014). Tolerance to drought and salt stress in plants: unraveling the signaling networks. *Frontiers in plant science*, *5*, 151. doi: 10.3389/fpls.2014.00151.

Goussi, R., Manaa, A., Derbali, W., Cantamessa, S., Abdelly, C., & Barbato, R. (2018). Comparative analysis of salt stress, duration and intensity, on the chloroplast ultrastructure and photosynthetic apparatus in Thellungiella salsuginea. *Journal of Photochemistry and Photobiology B: Biology*, *183*, 275-287.

Gupta S, Seth R, Sharma A (2016) Plant growth-promoting rhizobacteria play a pole as phytostimulators for sustainable agriculture. In: Choudhary DK et al (eds) Plant-microbe interaction: an approach to sustainable agriculture. Springer, Singapore, pp 475–493. https://doi.org/10.1007/978-981-10-2854-0_22

Gupta, A., Bano, A., Rai, S., Mishra, R., Singh, M., Sharma, S., & Pathak, N. (2022). Mechanistic insights of plant-microbe interaction towards drought and salinity stress in plants for enhancing the agriculture productivity. *Plant Stress*, *4*, 100073.doi: 10.1016/j.stress.2022.100073

Gupta, S., Schillaci, M., Walker, R., Smith, P.M.C., Watt, M. & Roessner, U. (2020). Alleviation of salinity stress in plants by endophytic plant-fungal symbiosis: current knowledge, perspectives and future directions. *Plant Soil*, 461, 219–244. https://doi.org/10.1007/s11104-020-04618-w

Haider, M. W., Nafees, M., Ahmad, I., Ali, B., Maryam, Iqbal, R., & Elshikh, M. S. (2022). Postharvest dormancy-related changes of endogenous hormones in relation to different dormancy-breaking methods of potato (*Solanum tuberosum* L.) tubers. *Frontiers in Plant Science*, *13*, 945256.

Hajiboland, R., Aliasgharzadeh, N., Laiegh, S.F., & Poschenrieder, C. (2010). Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. *Plant Soil*, 331, 313-327. https://doi.org/10.1007/s11104-009-0255-z

Hamayun, M., Khan, S. A., Khan, A.L., Rehman, G., Kim, Y. H., Iqbal, I., & Lee, I.J. (2010). Gibberellin production and plant growth promotion from pure cultures of *Cladosporium* sp. MH-6 isolated from cucumber (*Cucumis sativus* L.). *Mycologia*, 102(5), 989-995. https://doi.org/10.3852/09-261

Hameed, A., Ahmed, M.Z., Hussain, T., Aziz, I., Ahmad, N., Gul, B., & Nielsen, B.L. (2021). Effects of salinity stress on chloroplast structure and function. *Cells*, *10*(8), 2023.

Hasanuzzaman, M., & Fujita, M. (2022). Plant responses and tolerance to salt stress: Physiological and molecular interventions. *International Journal of Molecular Sciences*, *23*(9), 4810. https://doi.org/ 10.3390/ijms23094810

Hasanuzzaman, M., Bhuyan, M.B., Anee, T.I., Parvin, K., Nahar, K., Mahmud, J.A., & Fujita, M. (2019). Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. *Antioxidants*, *8*(9), 384.

Hasanuzzaman, M., Raihan, M.R.H., Masud, A.A.C., Rahman, K., Nowroz, F., Rahman, M., & Fujita, M. (2021). Regulation of reactive oxygen species and antioxidant defense in plants under salinity. *International Journal of Molecular Sciences*, *22*(17), 9326. doi: 10.3390/ijms22179326

Hashem, A., Abd_Allah, E.F., Alqarawi, A. A., Al Huqail, A. A., & Egamberdieva, D. (2014). Alleviation of abiotic salt stress in Ochradenus baccatus (Del.) by *Trichoderma hamatum* (Bonord.) Bainier. *Journal Plant Interactions*, 9(1), 857-868. https://doi.org/10.1080/17429145.2014.983568

Hossain, M.A., Hoque, M.A., Burritt, D.J., & Fujita, M. (2014). Proline protects plants against abiotic oxidative stress: biochemical and molecular mechanisms. In *Oxidative damage to plants* (pp. 477-522). Academic press. https://doi.org/10.1016/B978-0-12-799963-0.00016-2

Huang, H., Ullah, F., Zhou, D. X., Yi, M., & Zhao, Y. (2019). Mechanisms of ROS regulation of plant development and stress responses. *Frontiers in plant science*, *10*, 800.

Huchzermeyer, B., Menghani, E., Khardia, P. & Shilu, A. (2022). Metabolic pathway of natural antioxidants, antioxidant enzymes and ROS providence. *Antioxidants,* 11(4), 761. https://doi.org/10.3390/antiox11040761

Huihui, Z., Yue, W., Xin, L., Guoqiang, H., Yanhui, C., Zhiyuan, T., & Guangyu, S. (2020). Chlorophyll synthesis and the photoprotective mechanism in leaves of mulberry (*Morus alba* L.) seedlings under NaCl and NaHCO3 stress revealed by TMT-based proteomics analyses. *Ecotoxicology and Environmental Safety*, *190*, 110164.

Isayenkov, S.V. & Maathuis, F. J. (2019). Plant salinity stress: many unanswered questions remain. *Frontiers Plant Science*, 10, 80. https://doi.org/10.3389/fpls.2019.00080.

Jamil, F., Mukhtar, H., Fouillaud, M. & Dufoss´e, L. (2022). Rhizosphere signaling: Insights into plant-rhizomicrobiome interactions for sustainable agronomy. *Microorganisms*, 10, 899. https://doi.org/10.3390/microorganisms10050899

Jiang, H., Qi, P., Wang, T., Chi, X., Wang, M., Chen, M., & Pan, L. (2019). Role of halotolerant phosphate-solubilising bacteria on growth promotion of peanut (*Arachis hypogaea*) under saline soil. *Annals of Applied Biology*, *174*(1), 20-30.

Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., Chattha, M.S., & Chen, J. T. (2019). An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. *International journal of molecular sciences*, *21*(1), 148. doi: 10.3390/ijms21010148

Kashyap, P.L., Srivastava, A.K., Tiwari, S.P. & Kumar, S. (2018). Microbes for Climate Resilient Agriculture; John Wiley & Sons: Hoboken, NJ, USA

Kaya, C., Ashraf, M., Sonmez, O., Aydemir, S., Tuna, A.L., & Cullu, M.A. (2009). The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. *Scientia Horticulturae*, 121(1), 1-6. https://doi.org/10.1016/j.scienta.2009.01.001

Khademian, R., Asghari, B., Sedaghati, B., & Yaghoubian, Y. (2019). Plant beneficial rhizospheric microorganisms (PBRMs) mitigate deleterious effects of salinity in sesame (*Sesamum indicum* L.): physio-biochemical properties, fatty acids composition and secondary metabolites content. *Industrial Crops Products*, 136, 129–139. https://doi.org/ 10.1016/j.indcrop.2019.05.002.

Khalid, M., Hassani, D., Liao, J., Xiong, X., Bilal, M., & Huang, D. (2018). An endosymbiont *Piriformospora indica* reduces adverse effects of salinity by regulating cation transporter genes, phytohormones, and antioxidants in *Brassica campestris* ssp. *chinensis*. *Environmental Experimental Botany*, 153, 89-99. https://doi.org/10.1016/j.envexpbot.2018.05.007

Khalvandi, M., Amerian, M., Pirdashti, H., Keramati, S., & Hosseini, J. (2019). Essential oil of peppermint in symbiotic relationship with *Piriformospora indica* and methyl jasmonate application under saline condition. *Industrial Crops Products*, 127, 195-202. https://doi.org/10.1016/j.indcrop.2018.10.072

Khatun, S., & Flowers, T.J. (1995). Effects of salinity on seed set in rice. *Plant, Cell & Environment*, *18*(1), 61-67.

Khomari, S., Golshan-Doust, S., Seyed-Sharifi, R., & Davari, M. (2018). Improvement of soybean seedling growth under salinity stress by biopriming of high-vigour seeds with salt-tolerant isolate of *Trichoderma harzianum*. *New Zealand Journal Crop Horticultural Science*, 46(2), 117-132. https://doi.org/10.1080/01140671.2017.1352520

Khushdil, F., Jan, F. G., Jan, G., Hamayun, M., Iqbal, A., Hussain, A., & Bibi, N. (2019). Salt stress alleviation in *Pennisetum glaucum* through secondary metabolites modulation by Aspergillus terreus. *Plant Physiology Biochemistry*, 144, 127-134. https://doi.org/10.1016/j.plaphy.2019.09.038

Kohler, J., Hernández, J.A., Caravaca, F., & Roldán, A. (2009). Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. *Environmental Experimental Botany*, 65(2-3), 245-252. https://doi.org/10.1016/j.envexpbot.2008.09.008

Korkom, Y. (2023). Drought Stress is a Global Problem in Sustainable Agriculture: Response of Plant, Role of Fungal Endophytes. In Türker, H. B. & Gül, A. (Eds.). Architectural Sciences and Urban Agriculture II. 2023, Chapter: 8, 223-254. ISBN: 978-625-367-299-7. Iksad Publications. https://doi.org/10.5281/zenodo.8385856

Krings, M., Taylor, T.N., Hass, H., Kerp, H., Dotzler, N., & Hermsen, E. J. (2007). Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. *New Phytologist*, 174(3), 648-657. https://doi.org/10.1111/j.1469-8137.2007.02008.x

Krishnamurthy, P., Qingsong, L., & Kumar, P.P. (2018). Proteomics perspectives in post-genomic era for producing salinity stress-tolerant crops. *Salinity Responses and Tolerance in Plants, Volume 2: Exploring RNAi, Genome Editing and Systems Biology*, 239-266.

Kumar, A., & Verma, J.P. (2018). Does plant-Microbe interaction confer stress tolerance in plants: a review? *Microbiological Research*, 207, 41–52. https://doi.org/10.1016/j. micres.2017.11.004.

Kumar, P. & Sharma, P.K. (2020). Soil salinity and food security in India. *Frontiers Sustainable Food Systems*, 4, 533781. https://doi.org/10.3389/fsufs.2020.533781

Kumari S, Varma A, Tuteja N, Choudhary DK (2016) Bacterial ACC-deaminase: An eco-friendly strategy to cope abiotic stresses for sustainable agriculture. In: Choudhary DK et al (eds) Plant-microbe interaction: an approach to sustainable agriculture. Springer, Singapore, pp 165–185. https://doi. org/10.1007/978-981-10-2854-0_8

Kumari, A., Das, P., Parida, A.K., & Agarwal, P.K. (2015). Proteomics, metabolomics, and ionomics perspectives of salinity tolerance in halophytes. *Frontiers in Plant Science*, *6*, 537.

Kushwaha, P., Kashyap, P.L., Bhardwaj, A.K., Kuppusamy, P., Srivastava, A. K., & Tiwari, R. K. (2020). Bacterial endophyte mediated plant tolerance to salinity: growth responses and mechanisms of action. *World Journal of Microbiology and Biotechnology*, *36*, 1-16. https://doi.org/10.1007/s11274-020-2804-9

Lata, R., Chowdhury, S., Gond, S. K. & White, J.F. (2018). Induction of abiotic stress tolerance in plants by endophytic microbes. *Letters Applied Microbiology*, *66*(4), 268–276. https://doi.org/10.1111/lam.12855.

Li, H.Q., & Jiang, X.W. (2017). Inoculation with plant growth-promoting bacteria (PGPB) improves salt tolerance of maize seedling. *Russian Journal of Plant Physiology*, *64*, 235-241.

Liu, S., Tian, Y., Jia, M., Lu, X., Yue, L., Zhao, X., & Wang, R. (2020). Induction of salt tolerance in *Arabidopsis thaliana* by volatiles from *Bacillus amyloliquefaciens* FZB42 via the jasmonic acid signaling pathway. *Frontiers in Microbiology*, *11*, 562934.

Ma, Q., Yue, L. J., Zhang, J. L., Wu, G. Q., Bao, A. K., & Wang, S. M. (2012). Sodium chloride improves photosynthesis and water status in the succulent xerophyte *Zygophyllum xanthoxylum*. *Tree Physiology*, *32*(1), 4-13. DOI: 10.1093/treephys/tpr098.

Ma, Y., Dias, M. C., & Freitas, H. (2020). Drought and salinity stress responses and microbe-induced tolerance in plants. *Frontiers in Plant Science*, *11*, 591911. doi: 10.3389/fpls.2020.591911

Ma, Y., Rajkumar, M., Zhang, C., & Freitas, H. (2016). Beneficial role of bacterial endophytes in heavy metal phytoremediation. *Journal of environmental management*, *174*, 14-25.

Magallon, K.J., & Dinneny, J.R. (2019). Environmental stress: Salinity ruins a plant’s day in the sun. *Current Biology*, *29*(10), R360-R362.

Marriboina, S., & Attipalli, R.R. (2020). Hydrophobic cell-wall barriers and vacuolar sequestration of Na+ ions are among the key mechanisms conferring high salinity tolerance in a biofuel tree species, *Pongamia pinnata* L. pierre. *Environmental and Experimental Botany*, *171*, 103949.

Mastouri, F., Björkman, T., & Harman, G.E. (2010). Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathology*, 100(11), 1213-1221. https://doi.org/10.1094/PHYTO-03-10-0091

Mbarki, S., Cerdà, A., Brestic, M., Mahendra, R., Abdelly, C., & Pascual, J.A. (2017). Vineyard compost supplemented with *Trichoderma harzianum* T78 improve saline soil quality. *Land Degradation Development*, 28(3), 1028-1037. https://doi.org/10.1002/ldr.2554

Meena, M., Swapnil, P., Zehra, A., Aamir, M., Dubey, M.K., Goutam, J., & Upadhyay, R. S. (2017). Beneficial microbes for disease suppression and plant growth promotion. In: Singh, D., Singh, H., Prabha, R. (eds) Plant-Microbe Interactions in Agro-Ecological Perspectives. Springer. https://doi.org/10.1007/978-981-10-6593-4_16

Meng, X., Yan, D., Long, X., Wang, C., Liu, Z., & Rengel, Z. (2014). Colonization by endophytic *Ochrobactrum anthropi* Mn1 promotes growth of Jerusalem artichoke. *Microbial biotechnology*, *7*(6), 601-610.

Metwally, R.A., & Soliman, S.A. (2023). Alleviation of the adverse effects of NaCl stress on tomato seedlings (*Solanum lycopersicum* L.) by *Trichoderma viride* through the antioxidative defense system. *Botanical Studies*, 64(1), 1-18. https://doi.org/10.1186/s40529-023-00368-x

Miliute, I., Buzaite, O., Baniulis, D., & Stanys, V. (2015). Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. *Zemdirbyste-Agriculture*, *102*(4), 465-478. https://doi.org/10.13080/z-a.2015.102.060

Miller, G.A.D., Suzuki, N., Ciftci-Yilmaz, S.U.L.T.A.N., & Mittler, R.O.N. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, cell & environment*, *33*(4), 453-467.

Minhas, P. S. & Dagar, J.C. (2016). Use of tree plantations in water-table drawdown and combating soil salinity. In: Dagar, J.C., Minhas, P. (Eds.), Agroforestry for the Management of Waterlogged Saline Soils and Poor-Quality Waters. Springer, India, New Delhi, pp. 33–48.

Mittler, R., & Blumwald, E. (2015). The roles of ROS and ABA in systemic acquired acclimation. *The Plant Cell*, *27*(1), 64-70. doi: 10.1105/tpc.114.133090.

Monetti, E., Kadono, T., Tran, D., Azzarello, E., Arbelet-Bonnin, D., Biligui, B., & Bouteau, F. (2014). Deciphering early events involved in hyperosmotic stress-induced programmed cell death in tobacco BY-2 cells. *Journal Experimental Botany,* 65(5), 1361-1375. https://doi.org/10.1093/jxb/ert460

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, cell & environment*, *25*(2), 239-250.

Munns, R. (2011). Plant adaptations to salt and water stress: differences and commonalities. *Advances in botanical research*, *57*, 1-32.

Munns, R., & Gilliham, M. (2015). Salinity tolerance of crops–what is the cost?. *New phytologist*, *208*(3), 668-673.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, *59*, 651-681.

Najar, R., Aydi, S., Sassi-Aydi, S., Zarai, A., & Abdelly, C. (2019). Effect of salt stress on photosynthesis and chlorophyll fluorescence in Medicago truncatula. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, *153*(1), 88-97. DOI: 10.1080/11263504.2018.1461701.

Numan, M., Bashir, S., Khan, Y., Mumtaz, R., Shinwari, Z.K., Khan, A. L., & Ahmed, A. H. (2018). Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. *Microbiological research*, *209*, 21-32.https://doi.org/10.1016/j.micres.2018.02.003

Oteino, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J., & Dowling, D.N. (2015). Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. *Frontiers in microbiology*, *6*, 745.

Oukala, N., Aissat, K., & Pastor, V. (2021). Bacterial endophytes: The hidden actor in plant immune responses against biotic stress. *Plants*, *10*(5), 1012.

Padikasan, I. A., Chinnannan, K., Kumar, S., & Subramaniyan, G. (2018). Agricultural biotechnology: engineering plants for improved productivity and quality. In *Omics Technologies and Bio-Engineering* (pp. 87-104). Academic Press.

Pandey PK, Yadav SK, Singh A, Sarma BK, Mishra A, Singh HB (2012) Cross-species alleviation of biotic and abiotic stresses by the endophyte *Pseudomonas aeruginosa* PW09. J Phytopathol 160:532–539

Parihar, P., Singh, S., Singh, R., Singh, V.P., & Prasad, S.M. (2015). Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental science and pollution research*, *22*, 4056-4075. DOI 10.1007/s11356-014-3739-1

Parul Parihar & Samiksha Singh & Rachana Singh & Vijay Pratap Singh & Sheo Mohan Prasad Effect of salinity stress on plants and its tolerance strategies: a review. Environ Sci Pollut Res (2015) 22:4056–4075 DOI 10.1007/s11356-014-3739-1

Parvin, K., Ahamed, K.U., Islam, M.M., & Haque, N. (2016). Modulation of ion uptake in tomato (*Lycopersicon esculentum* L.) plants with exogenous application of calcium under salt stress condition. *Poljoprivreda*, *22*(2), 40-49.

Pedranzani, H., Racagni, G., Alemano, S., Miersch, O., Ramírez, I., Peña-Cortés, H., ... & Abdala, G. (2003). Salt tolerant tomato plants show increased levels of jasmonic acid. *Plant Growth Regulation*, *41*, 149-158.

Phetcharat, P., & Duangpaeng, A. (2012). Screening of endophytic bacteria from organic rice tissue for indole acetic acid production. *Procedia Engineering*, *32*, 177-183.

Pistelli, L., Libik-Konieczny, M., & Hasanuzzaman, M. (2023). Plant-microbe interactions and their role in salinity tolerance. *Frontiers in Plant Science*, *14*, 1142563. doi: 10.3389/fpls.2023.1142563

Porcel, R., Redondo-Gómez, S., Mateos-Naranjo, E., Aroca, R., Garcia, R., & Ruiz-Lozano, J.M. (2015). Arbuscular mycorrhizal symbiosis ameliorates the optimum quantum yield of photosystem II and reduces non-photochemical quenching in rice plants subjected to salt stress. *Journal Plant Physiology*, 185, 75-83. https://doi.org/10.1016/j.jplph.2015.07.006

Prakash, L., & Prathapasenan, G. (1990). NaCl-and gibberellic acid-induced changes in the content of auxin and the activities of cellulase and pectin lyase during leaf growth in rice (*Oryza sativa*). *Annals of Botany*, *65*(3), 251-257.

Qin, Y., Druzhinina, I.S., Pan, X., & Yuan, Z. (2016). Microbially mediated plant salt tolerance and microbiome-based solutions for saline agriculture. *Biotechnology Advances*, *34*(7), 1245-1259.

Rahman, A., Nahar, K., Hasanuzzaman, M., & Fujita, M. (2016). Calcium supplementation improves Na+/K+ ratio, antioxidant defense and glyoxalase systems in salt-stressed rice seedlings. *Frontiers in plant science*, *7*, 609. doi: 10.3389/fpls.2016.00609

Rath, K.M., Maheshwari, A., & Rousk, J. (2019). Linking microbial community structure to trait distributions and functions using salinity as an environmental filter. *Mbio*, *10*(4), 10-1128.

Rawat, L., Singh, Y., Shukla, N., & Kumar, J. (2011). Alleviation of the adverse effects of salinity stress in wheat (*Triticum aestivum* L.) by seed biopriming with salinity tolerant isolates of *Trichoderma harzianum*. *Plant Soil*, 347, 387-400. https://doi.org/10.1007/s11104-011-0858-z

Rawat, L., Singh, Y., Shukla, N., & Kumar, J. (2012). Seed biopriming with salinity tolerant isolates of *Trichoderma harzmnum* alleviates salt stress in rice: growth, physiological and biochemical characteristics. *Journal Plant Pathology*, 353-365. https://www.jstor.org/stable/45156044

Redman, R.S., Freeman, S., Clifton, D.R., Morrel, J., Brown, G., Rodriguez, R.J. (1999) Biochemical analysis of plant protection afforded by a non-pathogenic endophytic mutant of *Colletotrichum magna*. *Plant Physiol 119*:795–804

Rejeb, I.B., Pastor, V., Mauch-Mani, B. (2014). Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. *Plants (Basel)*, 3(4), 458–475. https://doi. org/10.3390/plants3040458

Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J., & Dowling, D.N. (2008). Bacterial endophytes: recent developments and applications. *FEMS microbiology letters*, *278*(1), 1-9.

Santander, C., Ruiz, A., García, S., Aroca, R., Cumming, J., & Cornejo, P. (2020). Efficiency of two arbuscular mycorrhizal fungal inocula to improve saline stress tolerance in lettuce plants by changes of antioxidant defense mechanisms. *Journal Science Food Agriculture*, 100(4), 1577-1587. https://doi.org/10.1002/jsfa.10166

Sarkar, A.; Pramanik, K.; Mitra, S.; Soren, T.; Maiti, T.K. Enhancement of Growth and Salt Tolerance of Rice Seedlings by ACC Deaminase-Producing *Burkholderia* sp. MTCC 12259. *J. Plant Physiol.* 2018, *231*, 434–442.

Schulz B, Boyle C, Draeger S, Rommert AK, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 109:996–1004

Sharma, P., Jha, A.B., Dubey, R.S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of botany*, *2012*.

Sharma, P., Kharkwal, A.C., Abdin, M.Z., & Varma, A. (2017). *Piriformospora indica*-mediated salinity tolerance in *Aloe vera* plantlets. *Symbiosis*, 72, 103-115. https://doi.org/10.1007/s13199-016-0449-0

Sheldon, A.R., Dalal, R.C., Kirchhof, G., Kopittke, P.M., & Menzies, N. W. (2017). The effect of salinity on plant-available water. *Plant and Soil*, *418*, 477-491.

Shi, Y., Yang, H., Zhang, T., Sun, J., & Lou, K. (2014). Illumina-based analysis of endophytic bacterial diversity and space-time dynamics in sugar beet on the north slope of Tianshan mountain. *Applied microbiology and biotechnology*, *98*, 6375-6385.

Singh, D. (2022). Juggling with reactive oxygen species and antioxidant defense system – A coping mechanism under salt stress. *Plant Stress,* 5, 100093. https://doi.org/10.1016/j.stress.2022.100093

Singh, M., Kumar, J., Singh, S., Singh, V.P., & Prasad, S.M. (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. *Reviews in Environmental Science and Bio/Technology*, *14*, 407-426.

Siringam, K., Juntawong, N., Cha-um, S., & Kirdmanee, C. (2011). Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt-sensitive rice (*Oryza sativa* L. spp. *indica*) roots under isoosmotic conditions. *African Journal of Biotechnology*, *10*(8), 1340-1346.

Slama, H. B., Cherif-Silini, H., Chenari Bouket, A., Qader, M., Silini, A., Yahiaoui, B., & Belbahri, L. (2019b). Screening for *Fusarium* antagonistic bacteria from contrasting niches designated the endophyte

Bacillus halotolerans as plant warden against Fusarium. *Frontiers in microbiology*, *9*, 3236.

Slama, H.B, Triki, M.A., Chenari Bouket, A., Ben Mefteh, F., Alenezi, F.N., Luptakova, L., & Belbahri, L. (2019a). Screening of the high-rhizosphere competent *Limoniastrum monopetalum*' culturable endophyte microbiota allows the recovery of multifaceted and versatile biocontrol agents. *Microorganisms*, *7*(8), 249.

Slama, H.B.; Chenari Bouket, A.; Alenezi, F.N.; Luptakova, L.; Baranov, O.; Ahadi, R.; Belbahri, L. (2023). Impacts of Salt Stress on the Rhizosphere and Endophytic Bacterial Role in Plant Salt Alleviation. Int. J. Plant Biol. 2023, 14, 361–376. https://doi.org/ 10.3390/ijpb14020030

Solanki, M.K., Solanki, A.C., Rai, S., Srivastava, S., Kashyap, B.K., Divvela, P.K., & Qureshi, K.A. (2022). Functional interplay between antagonistic bacteria and *Rhizoctonia solani* in the tomato plant rhizosphere. *Frontiers in Microbiology*, *13*, 990850.

Soliman, M.H., Alnusaire, T.S., Abdelbaky, N.F., Alayafi, A. A., Hasanuzzaman, M., Rowezak, M.M., & Elkelish, A. (2020). *Trichoderma*-induced improvement in growth, photosynthetic pigments, proline, and glutathione levels in *Cucurbita pepo* seedlings under salt stress. *Phyton*, 89(3), 473. https://doi.org/10.32604/phyton.2020.08795

Stepien, P., & Johnson, G.N. (2009). Contrasting responses of photosynthesis to salt stress in the glycophyte *Arabidopsis* and the halophyte *Thellungiella*: role of the plastid terminal oxidase as an alternative electron sink. *Plant physiology*, *149*(2), 1154-1165. DOI: 10.1104/pp.108.132407.

Stringlis, I.A., Proietti, S., Hickman, R., Van Verk, M.C., Zamioudis, C., & Pieterse, C.M. (2018). Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune elicitors reveal signatures of adaptation to mutualists. *The Plant Journal*, *93*(1), 166-180.

Sun, L., Lei, P., Wang, Q., Ma, J., Zhan, Y., Jiang, K., & Xu, H. (2020). The endophyte *Pantoea alhagi* NX-11 alleviates salt stress damage to rice seedlings by secreting exopolysaccharides. *Frontiers in microbiology*, *10*, 3112.

Talaat, N.B., & Shawky, B.T. (2014). Modulation of the ROS-scavenging system in salt-stressed wheat plants inoculated with arbuscular mycorrhizal fungi. *Journal of Plant Nutrition Soil Science*, 177(2), 199-207. https://doi.org/10.1002/jpln.201200618

Tamosiune, I., Baniulis, D., & Stanys, V. (2017). Role of endophytic bacteria in stress tolerance of agricultural plants: diversity of microorganisms and molecular mechanisms. *Probiotics in agroecosystem*, 1-29. https://doi.org/10.1007/978-981-10-4059-7_1

Tsonev, T.D., Lazova, G.N., Stoinova, Z.G., & Popova, L.P. (1998). A possible role for jasmonic acid in adaptation of barley seedlings to salinity stress. *Journal of Plant Growth Regulation*, *17*, 153-159.

Tufail, M.A., Bejarano, A., Shakoor, A., Naeem, A., Arif, M. S., Dar, A.A., & Puopolo, G. (2021). Can bacterial endophytes be used as a promising bio-inoculant for the mitigation of salinity stress in crop plants? A global meta-analysis of the last decade (2011–2020). *Microorganisms*, *9*(9), 1861. https://doi.org/ 10.3390/microorganisms9091861

Ullah, A., Manghwar, H., Shaban, M., Khan, A.H., Akbar, A., Ali, U., & Fahad, S. (2018). Phytohormones enhanced drought tolerance in plants: a coping strategy. *Environmental Science and Pollution Research*, *25*, 33103-33118. doi: 10.1007/s11356-018-3364-5

Vaishnav, A., Shukla, A.K., Sharma, A., Kumar, R., & Choudhary, D. K. (2019). Endophytic bacteria in plant salt stress tolerance: current and future prospects. *Journal of Plant Growth Regulation*, *38*, 650-668.

Verma, P., Yadav, A.N., Khannam, K.S., Kumar, S., Saxena, A.K., & Suman, A. (2016). Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. *Journal of Basic Microbiology*, *56*(1), 44-58.

Verma, S.K., Kingsley, K., Irizarry, I., Bergen, M., Kharwar, R.N., & White Jr, J.F. (2017). Seed-vectored endophytic bacteria modulate development of rice seedlings. *Journal of Applied Microbiology*, *122*(6), 1680-1691.

Volkmar, K.M., Hu, Y., & Steppuhn, H. (1998). Physiological responses of plants to salinity: a review. *Canadian journal of plant science*, *78*(1), 19-27.

Wang, L., Xu, J. Y., Jia, W., Chen, Z., & Xu, Z.C. (2020). Chloride salinity in a chloride-sensitive plant: Focusing on photosynthesis, hormone synthesis and transduction in tobacco. *Plant physiology and biochemistry*, *153*, 119-130. DOI: 10.1016/j.plaphy.2020.05.021.

Wang, Y., Mopper, S., & Hasenstein, K.H. (2001). Effects of salinity on endogenous ABA, IAA, JA, and SA in Iris hexagona. *Journal of chemical ecology*, *27*, 327-342.

Waqas, M., Khan, A.L., Kamran, M., Hamayun, M., Kang, S. M., Kim, Y.H., & Lee, I.J. (2012). Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules*, 17(9), 10754-10773. https://doi.org/10.3390/molecules170910754

Watts, D.; Palombo, E.A.; Jaimes Castillo, A.; Zaferanloo, B. Endophytes in Agriculture: Potential to Improve Yields and Tolerances of Agricultural Crops. Microorganisms 2023, 11, 1276. https://doi.org/ 10.3390/microorganisms11051276

Wu, L., Huo, W., Yao, D., & Li, M. (2019). Effects of solid matrix priming (SMP) and salt stress on broccoli and cauliflower seed germination and early seedling growth. *Scientia Horticulturae*, *255*, 161-168.

Xu, L., Wu, C., Oelmuller, R., & Zhang, W. (2018). Role of phytohormones in *Piriformospora indica*-induced growth promotion and stress tolerance in plants: more questions than answers. *Frontiers in Microbiology*, 9, 1646. https://doi.org/10.3389/fmicb.2018.01646.

Yadav, A.N., Kaur, T., Kour, D., Rana, K.L., Yadav, N., Rastegari, A.A., Kumar, M., Paul, D., Sachan, S.G., & Saxena, A.K. (2020). Saline microbiome: Biodiversity, ecological significance, and potential role in amelioration of salt stress. In *New and Future Developments in Microbial Biotechnology and Bioengineering*; Rastegari, A.A., Yadav, A.N., Yadav, N., Eds.; Elsevier: Amsterdam, The Netherlands, 283–309.

Yadav, S. P., Bharadwaj, R., Nayak, H., Mahto, R., Singh, R. K., & Prasad, S. K. (2019). Impact of salt stress on growth, productivity and physicochemical properties of plants: A Review. *Int. J. Chem. Stud*, *7*(2), 1793-1798.

Yaish, M.W. (2017). Draft genome sequence of the endophytic *Bacillus aryabhattai* strain SQU-R12, identified from Phoenix dactylifera L. roots. *Genome Announcements*, *5*(32), 10-1128.

Yang, B., Wang, X.M., Ma, H.Y., Yang, T., Jia, Y., Zhou, J., & Dai, C. C. (2015). Fungal endophyte *Phomopsis liquidambari* affects nitrogen transformation processes and related microorganisms in the rice rhizosphere. *Frontiers Microbiology*, 6, 982. https://doi.org/10.3389/fmicb.2015.00982

Yang, L., Wen, K.S., Ruan, X., Zhao, Y.X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, 23(4), 762. https://doi.org/10.3390/molecules23040762

Yavaş, İ., & İlker, E. (2020). Çevresel stres koşullarına maruz kalan bitkilerde fotosentez ve fitohormon seviyelerindeki değişiklikler. *Bahri Dağdaş Bitkisel Araştırma Dergisi*, *9*(2), 295-311.

Yildiz, M., Poyraz, İ., Çavdar, A., Özgen, Y., & Beyaz, R. (2020). Plant responses to salt stress. *Plant breeding-current and future views*. doi: 10.5772/intechopen.93920

Yun, P., Xu, L., Wang, S.S., Shabala, L., Shabala, S., & Zhang, W.Y. (2018*)*. *Piriformospora indica* improves salinity stress tolerance in *Zea mays* L. plants by regulating Na+ and K+ loading in root and allocating K+ in shoot. *Plant Growth Regulation*, 86, 323-331. https://doi.org/10.1007/s10725-018-0431-3

Yusnawan, E., Taufiq, A., Wijanarko, A., Susilowati, D. N., Praptana, R.H., Chandra-Hioe, M. V., & Inayati, A. (2021). Changes in volatile organic compounds from salt-tolerant *Trichoderma* and the biochemical response and growth performance in saline-stressed groundnut. *Sustainability*, 13(23), 13226. https://doi.org/10.3390/su132313226

Zarea, M.J., Hajinia, S., Karimi, N., Goltapeh, E.M., Rejali, F., & Varma, A. (2012). Effect of *Piriformospora indica* and *Azospirillum* strains from saline or non-saline soil on mitigation of the effects of NaCl. *Soil Biology Biochemistry*, 45, 139-146. https://doi.org/10.1016/j.soilbio.2011.11.006

Zhang, Q., & Dai, W. (2019). Plant response to salinity stress. In *Stress physiology of woody plants* (pp. 155-173). CRC Press.

Zhang, S., Gan, Y., & Xu, B. (2016). Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Frontiers Plant Science*, 7, 1405. https://doi.org/10.3389/fpls.2016.01405

Zhang, S., Xu, B., & Gan, Y. (2019). Seed treatment with *Trichoderma longibrachiatum* T6 promotes wheat seedling growth under NaCl stress through activating the enzymatic and nonenzymatic antioxidant defense systems. *International Journal of Molecular Sciences*, 20(15), 3729. https://doi.org/10.3390/ijms20153729

Zhang, W.W., Chong, W.A.N.G., Rui, X.U.E., & Wang, L.J. (2019). Effects of salinity on the soil microbial community and soil fertility. *Journal of Integrative Agriculture*, *18*(6), 1360-1368. doi: 10.1016/S2095-3119(18)62077-5

Zhao, L., Wang, F., Zhang, Y., & Zhang, J. (2014). Involvement of *Trichoderma asperellum* strain T6 in regulating iron acquisition in plants. *Journal Basic Microbiology*, 54(S1), S115-S124. https://doi.org/10.1002/jobm.201400148

Zhao, S., Zhang, Q., Liu, M., Zhou, H., Ma, C., & Wang, P. (2021). Regulation of plant responses to salt stress. *International Journal of Molecular Sciences*, *22*(9), 4609.

Zhou, H., Shi, H., Yang, Y., Feng, X., Chen, X., Xiao, F., & Guo, Y. (2023). Insights into plant salt stress signaling and tolerance. *Journal of Genetics and Genomics*.

Zhu, B., Su, J., Chang, M., Verma, D. P. S., Fan, Y. L., & Wu, R. (1998). Overexpression of a Δ1-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water-and salt-stress in transgenic rice. *Plant Science*, *139*(1), 41-48.

Zhu, J.K (2016). Abiotic stress signaling and responses in plants. *Cell*, 167(2), 313-324. https://doi.org/10.1016/j.cell.2016.08.029.

Zhu, J.K. (2002). Salt and drought stress signal transduction in plants. *Annual review of plant biology*, *53*(1), 247-273.

Zörb, C., Geilfus, C.M., & Dietz, K.J. (2019). Salinity and crop yield. *Plant biology*, *21*, 31-38.

CHAPTER 8

THE RELATIONSHIP BETWEEN LEGUMES, *RHIZOBIUM* AND THE ENVIRONMENT

Nurdoğan TOPAL[1]

DOI: https://dx.doi.org/10.5281/zenodo.10450480

---

[1] Department Of Field Crops, Faculty of Agriculture, Uşak University, Uşak, Turkiye nurdogan.topal@usak.edu.tr Orcid ID 000-0002-3466-1005

INTRODUCTION

One issue that keeps getting worse is feeding the world's growing population. Due to their low cost of protein, edible legumes—one of the major plant groupings for human nutrition—are also referred to as "poor man's meat." Legume is becoming an increasingly significant component of all agricultural systems, whether they are conventional, organic, or good agriculture, as the need for agriculture to be sustainable grows. Legumes are special because of their symbiotic relationship with rhizobia bacteria, which allows them to benefit from the free nitrogen in the air in addition to their high nutritional content. Understanding the traits of the parties and how they interact with the environment is essential to getting the most out of this connection. It is covered in this article.

1. LEGUMES

Grain-based legumes have a important act in mankid nutrition, particularly for those with poor incomes in developing nations. (Tharanathan and Mahadevamma, 2003). Legumes are valuable to humans second only to the Gramineae. They are distinguished by their unusual flower shape, pod-like fruits, and the capacity of 88% of the species under investigation to create rhizobia-containing nodules. Legume species, which belong to the 670–750 genera and 18,000–19,000 species group, are crucial plants for grazing, agroforestry, and cereal crops (Graham and Vance, 2003). Grain legumes are being produced more widely in the world because to their direct use in industrial demands, animal feed, and human nutrition. Additionally, grain legumes can increase cropping systems' nitrogen and phosphorus contents (Sinclair and Vadez, 2012). Globally, grain legumes (including beans, broad beans, peas, lentils, chickpeas, and pigeon peas) are grown on 95,438,847 hectares with an average yield of 93.22 kg/da and an output of 88,966,861.44 tons, according to Fao statistics (2021). (FAO, 2023). Significant advancements have been made in the identification of novel bioactive chemicals and the development of functional food ingredients for the prevention of illness in recent years. It has long been believed that legumes are healthy diets for humans. Legumes are a family of plants whose seeds have long been a mainstay of human meals. Legumes are an excellent source of carbs, proteins, dietary fiber, vitamins, and minerals, but they also have a wide range of non-nutrient substances that are thought to be bioactive and have anticarcinogenic, hypoglycemia, hypoglycemic, and antioxidant qualities (Martín-Cabrejas, 2019). As important suppliers of plant protein, a number of grain legume crops are essential to world agriculture and nutrition, serving as both food and feed. When cultivated in agricultural rotations, legumes help to enhance the environment sustainably because of their ability to fix nitrogen biologically, their impacts on the soil, the yield of the following crop, and the services they provide to other agroecosystem components like pollinators (De Ron, 2015).

Rhizobia, or root-nodule relationships, are symbiotic with legumes (Dwivedi et al., 2015). Due to their symbiotic relationship with a class of soil bacteria known as rhizobia, legumes are able to absorb atmospheric N2 and so require less N fertilizer. Legumes in a rotation can result in less fertilizer N being used, as part of this "free" N is transferred to a next crop. Not to be overlooked are the additional advantages of legumes, such as decreased insect and weed incidence and enhanced soil quality (Van Kessel and Hartley, 2000).

2. RHIZOBIA (*Rhizobium*)

2.1. Taxonmy of Rhizobia

The fact that legume plants' root nodules were absorbing atmospheric nitrogen was discovered by the end of the 1800s. The rootnodule bacteria were isolated and identified as the cause of this nitrogen fixation process by Beijerinck in 1888. Despite several competing suggestions being given in the early years, Frank (1889) published the term Rhizobium leguminosarum after just one year. This is the name we still use today (Willems, 2006; Peter et al., 1996). Beijerinck combined the Greek terms "rhizo" and "bios," which mean "root" and "life," to create the nomenclature "Rhizobium." This was done to represent the bacteria's mutualistic relationship with plant roots (Allam, 2023).

Rhizobia are the bacteria that induce legumes to produce nitrogen-fixing nodules. Rhizobium is divided into four genera and 17 species. Rhizobia have been identified to have separated into 3 genera: *Rhizobium*, *Bradyrhizobium,* and *Azorhizobium* based on sequences found in small sub-unit ribosomal Ribo Nucleic Acid. These are distributed throughout several distinct branches, all of which contain a significant number of non-rhizobial bacterial species, and they are all members of the *Proteohacteria*'s alpha subdivision. *Rhizobium* continues to be vast and pholyphyletic, which is why there have lately been suggestions to split this genus into four genera (Peter et al., 1996).

Nodulation tests with a variety of bacteria and host plants were carried out in the beginning of the 20th century, and the specificity between the symbiotic bacteria and the host plants was noted. As a result, Baldwin and Fred developed the concept of cross-nodulation, which demonstrates the rhizobia's host plant selection. Taxonomists used this theory over a period of about eight decades to identify six main species: *R. lupini- R. japonicum* showed an alkaline reaction on yeast-extract mannitol agar (YMA) medium, whereas *R. meliloti - R. trifolii- R. phaseoli*, and *R. leguminosarum* displayed an acidic reaction. Those six species were not the only isolates from cowpea that were recognized as *Rhizobium* spp. (Helene et al., 2022). The last few years have seen a significant shift in the categorization of rhizobia as a result of the addition of many new genera and species to this significant bacterial group. The wide variety of nitrogen-fixing bacteria that have been isolated from various legumes has been demonstrated by recent investigations. Currently, 98 species of α-/β-*proteobacteria* belonging to 14 taxa have been identified as rhizobia. The group of bacteria known as legume symbionts includes the genera *Shinella, Ensifer* (formerly *Sinorhizobium*), Phyllobacterium, *Bradyrhizobium, Microvirga, Azorhizobium, Ocrhobactrum, Methylobacterium, Mezorhizobium*, *Devosia, Rhizobium* (Class of *α- proteobacteria*), *Cupriavidus* (formerly *Ralstonia*) *Burkholderia,* (Class of *β-proteobacteria*), and some *γ-proteobacteria*. Given that just 23% of all legumes have been discovered as having a symbiotic connection to date, there is undoubtedly still more to learn (Berrada and Fikri-Benbrahim, 2014). Agrobacterium's uniqueness, the recently proposed *Neorhizobium* genus, and the resurrection of *Allorhizobium* as a legitimate genus within the *Rhizobiaceae* are all supported by the phylogenomic research, which also raises the possibility that *R. giardinii* should be placed in a new genus. Data from in silico DNA–DNA hybridization (DDH) and estimations of average nucleotide identity (ANI) have been made available by genomics to define bacterial species boundaries (Ormeno-Orrillo et al., 2015).

-

Table 1. Taxonomy of *Rhizobium*

Kingdom	*Bacteria*
Phlum	*Proteobacteria*
Class	*Alphaproteobacteria*
Order	*Rhizobiales*
Family	*Rhizobiaceae*
Genus	*Rhizobium*

2.2. Structure of Rhizobia

Rhizobia, another name for nodule root bacteria, are rod-shaped, medium-sized bacteria with dimensions of 1.2–3.0 ~m in length and 0.5–0.9 ~m in breadth. They are made up of the slowly developing Bradyrhizobium spp. and the quickly growing Rhizobium spp. They are Gram-negative and don't make endospores. Rhizobia often show variable Gram staining, which varies according to the culture's age. While older, longer cells exhibit banding and contain unstained patches, younger cultures and nodular bacteroids frequently have uniform gram staining. It has been shown that the unstained patches are big polymeric beta-hydroxybutyric acid (PHBA) granules. Phase-contrast microscopy is used to illustrate the PHBA's refractile nature. The majority of rhizobia are aerobic chemoorganotrophs, making cultivation fairly simple. Younger cultures and nodular bacteroids often show homogenous gram staining, but older, longer cells show banding and contain unstained patches. The unstained regions have been recognized as sizable granules of polymeric beta-hydroxybutyric acid (PHBA). Hoben and Somasegaran (2012). Each creature has one to six flagella that help it move around. The majority of the studied species exhibit peritrichous insertion. Peritrichous organization also appears to present as polar or sub-polar insertion in strains derived from animals that have a one flagellum. (*Sinorhizobium fredii, R. mongolense, Sinorhizobium saheli Sinorhizobium terangae, and Sinorhizobium xinjiangense, R. galegae*). It is said that only *R. hainanense* exhibits clear polar flagellation (Kuykendall et al., 2015).

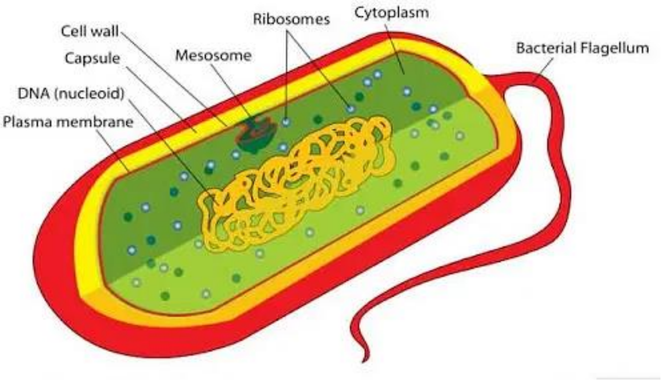
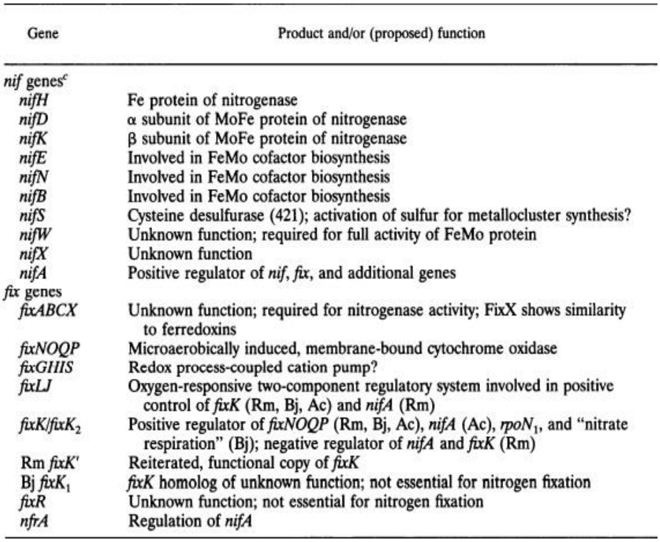


Figure 1. Structure of Rhizobium

2.3. Genetics of Rhizobia

The genomes of Rhizobium vary in size from 5.5 to 9 Mb. (Sessitsch et al., 2002). Nod, nif, and fix genes are the three categories into which symbiotic nitrogen fixation genes fall. Since the 20 *K. pneumoniae* nif genes and rhizobial nif genes are physically similar, it is assumed that a conserved nif gene functions similarly in both *K. pneumoniae* and rhizobia. Up to now, researchers have found at least nine distinct rhizobial nif genes in *A. Caulinodans, B. japonicum* and *R. meliloti,* (Table 2) (Fischer, 1994).

Table 2. Identification of the fix and nif genes in *R. meliloti, A. caulinodans*, or *B. japonicum* together with their suggested or known roles (Fischer, 1994).

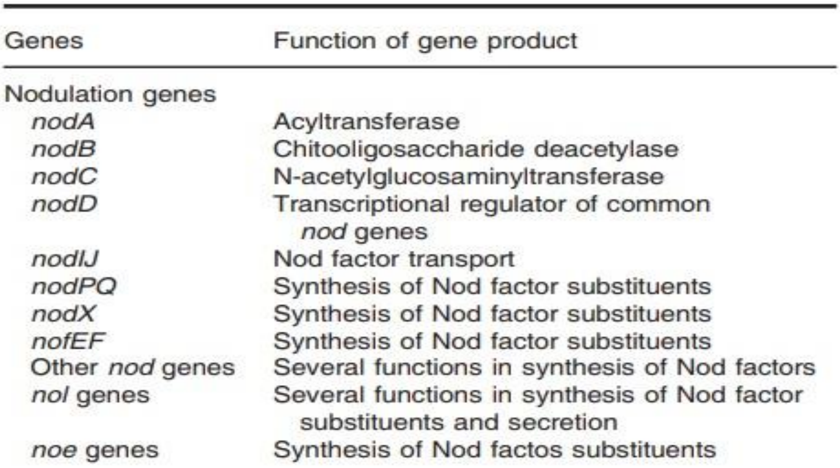


Gene	Product and/or (proposed) function
nif genes^c	
nifH	Fe protein of nitrogenase
nifD	α subunit of MoFe protein of nitrogenase
nifK	β subunit of MoFe protein of nitrogenase
nifE	Involved in FeMo cofactor biosynthesis
nifN	Involved in FeMo cofactor biosynthesis
nifB	Involved in FeMo cofactor biosynthesis
nifS	Cysteine desulfurase (421); activation of sulfur for metallocluster synthesis?
nifW	Unknown function; required for full activity of FeMo protein
nifX	Unknown function
nifA	Positive regulator of *nif*, *fix*, and additional genes
fix genes	
fixABCX	Unknown function; required for nitrogenase activity; FixX shows similarity to ferredoxins
fixNOQP	Microaerobically induced, membrane-bound cytochrome oxidase
fixGHIS	Redox process-coupled cation pump?
fixLJ	Oxygen-responsive two-component regulatory system involved in positive control of *fixK* (Rm, Bj, Ac) and *nifA* (Rm)
fixK/*fixK*$_2$	Positive regulator of *fixNOQP* (Rm, Bj, Ac), *nifA* (Ac), *rpoN*$_1$, and "nitrate respiration" (Bj); negative regulator of *nifA* and *fixK* (Rm)
Rm *fixK'*	Reiterated, functional copy of *fixK*
Bj *fixK*$_1$	*fixK* homolog of unknown function; not essential for nitrogen fixation
fixR	Unknown function; not essential for nitrogen fixation
nfrA	Regulation of *nifA*

The rhizobia belong to the α (alpha) and β (beta) class of proteobacteria, with genomes that are almost twice as large as those of typical bacteria. Each group's genome is split into two or more sizable replicons, one of which resembles the *Bacillus subtilis* or *Escherichia coli* major chromosome and contains many of the essential genes. Call them chromatids, plasmids, megaplasmids, or second chromosomes, they are the leftover replicons of one or more. Regarding *Sinorhizobium meliloti*, a 1.34 Mb megaplasmid has many SNF genes, such as fix, nif, and nod. Similar to this, plasmid or megaplasmid (pSym) containing SNF genes is present in other rhizobia species, such as *Burkholderia, Sinorhizobium, Phyllobacterium*, and. *Rhizobium* Symbiotic islands are areas where SNF genes are found in certain rhizobia species (*Azorhizobium, Mesorhizobium*, and *Bradyrhizobium*) that have a single big chromosome (Yousuf et al., 2022).

The nodulation (nod, noe, and nol) genes required for infection and nod ule organogenesis are present in all rhizobia that have been discovered to far. Certain genes, such nodABCD, are exclusive to certain species of rhizobia, whereas others are present in all of them. Sequence research showed that nod genes are highly conserved, even in closely related rhizobial lineages. This implies that the genes have been horizontally transmitted to several non-symbiotic bacterial species and may have originated from a monophyletic organism. For example, the nodD genes are transcriptional activators belonging to the lysR family. All rhizobia have a higher degree of genetic relatedness among their nodD genes than they do with any other lysR family member. The nodulation genes act as a mediator in the communication between rhizobia and legume host plants (Debellé et al., 2001).

Table 3. Nodulation genes and their Functions (Lindström and Mousavi, 2020).



Genes	Function of gene product
Nodulation genes	
nodA	Acyltransferase
nodB	Chitooligosaccharide deacetylase
nodC	N-acetylglucosaminyltransferase
nodD	Transcriptional regulator of common *nod* genes
nodIJ	Nod factor transport
nodPQ	Synthesis of Nod factor substituents
nodX	Synthesis of Nod factor substituents
nofEF	Synthesis of Nod factor substituents
Other *nod* genes	Several functions in synthesis of Nod factors
nol genes	Several functions in synthesis of Nod factor substituents and secretion
noe genes	Synthesis of Nod factos substituents

2.4. Life Cycle of Rhizobia

Legume plants and rhizobia, a kind of soil bacterium, can develop a symbiotic nitrogen-fixing partnership. Rhizobia exist in two stages: a free-living stage in the soil and a symbiotic phase in which they coexist with plants. They are symbionts that spread horizontally. Throughout their life cycle, rhizobia come into contact with a diverse array of microorganisms that alter their fitness and symbiotic performance (Agudelo et al., 2023).

It was discovered that Rhizobium strains with truncated or full life cycles were identified to originate from a diverse range of host plants. The former type, present in strains of garden flowers and some wild legumes, had septate and branched bacteroids within the nodule; both big, sometimes Gram-positive forms that resembled Bacillus and generated specialized, coceoid swarmers and resistant endospores, as well as tiny, Bacterium-like forms that infected the host plant, were among the free-living stages. (Bisset, 1952).

After the German scientist Beijerinck discovered rhizobia, Beijerinck noted in his research on pure bacterial culture that pure cultures consist of rod-like cells of 1-4 microns in size, coccoid cells of 0.18- 0.4 microns and bacteroids with large vacuoles. Bewley and Hutchinson (1920) reported that rhizobia proliferate by the development of coccoids inside of rod-like cells. They go over the five different kinds of cells and their life cycle sequence. Small non-motile coccoids are released by banded rods and grow bigger to become larger non-motile coccoids. These grow longer to become tiny, ovoid motile cells, which later transform into longer, motile rods without bands. The cycle is finished when the stainable material rounds off to create tiny coccoids. Although it was discovered that external factors affected the cocci's creation within the rods and changed their motile state, the development process was thought to be intrinsic and normal (Lewis,1938).

Rhizobia seem to gain a lot from symbiosis in terms of fitness. While rhizobial populations in soil seldom increase throughout years, a uniq rhizobial cell in a legume root nodule has the ability to replicate up to a million times. Rhizobial populations increased by 2.5 × 10^5 cells per g of soil during soybean nodule senescence. Cultivars that nodulate poorly with the target strain yield lower gains, indicating that soil population growth is not due to stimulation of rhizosphere populations but rather to rhizobial escape from nodules (Figure 2). When this increase is multiplied by 2 x 10^9 g soil (to plow depth) per hectare and divided by 2 x 10^5 plants per hectare, an estimated 2.5 × 10^9 rhizobia per plant are discharged. Each plant has twenty-five nodules, with one founder cell in each nodule. A few months after each rhizobial cell nodulates soybean, an estimated 10^8 offspring will emerge in the soil; this represents 10 to 100% of the 10^8 to 10^9 rhizobia in each soybean nodüle. (Denison and Kiers, 2011).

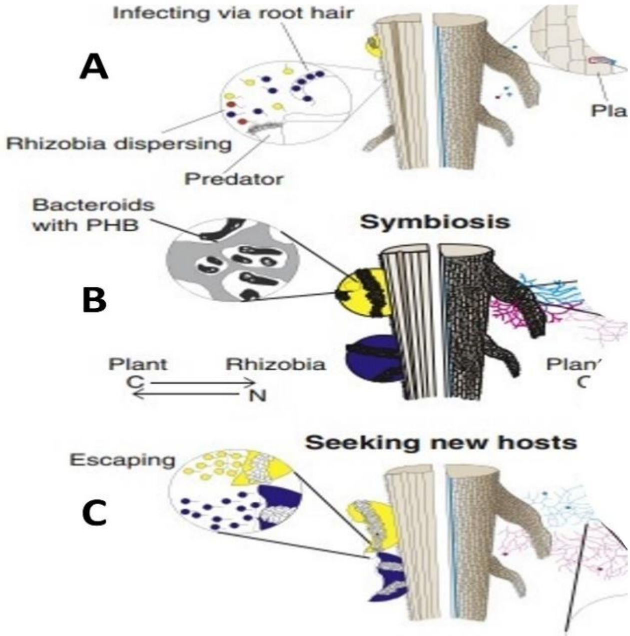


Figure 2. Life cycles of rhizobia (Denison and Kiers, 2011).

To show that there are several infection strains present in a single plant, different colors are assigned to each strain. (A) Top: Rhizobia usually infect hosts by root hairs, but occasionally they can also produce persisters in response to chemical cues from predators and high densities of competing populations. (B) Middle: To sustain N2 fixation, bacteroids that were once within root nodules must obtain carbon from plants. If too much carbon is diverted to stored polyhydroxybutyrate granules, a material that might increase rhizobial fitness, it could have negative effects on the host. (C) Bottom: Future competition between strains that break out from the same plant might undermine cooperation.

3. SYMBIOTIC NTROGEN FİXATİON İN LEGUMES

When the Papilionoideae (a subfamily of the Fabaceae) experienced genome duplication, the symbiosis first emerged, around 58 million years ago. Remarkably, the genes implicated in signaling appear to have been engaged in the symbiotic association with mycorrhiza at first. Therefore, it appears that the legumes were only able to initiate this nearly unique symbiotic interaction with rhizobia because of the whole genome duplication, which made genes accessible to carry out this novel role in communication (Bruning and Rozema 2013).

A successful completion of many phases is necessary to establish a completely functional symbiosis. These activities include the development and function of root nodules, the portion of the plant that fixes nitrogen, and the transfer of recognition signals between bacteria and plants. During the first stage of mutual sensing between the two species, the plant releases nutrients such as organic acids and amino acids along with flavonoids through its root exudates. Rhizobial nod genes are triggered by flavonoids secreted by the host plant into the rhizosphere. This initiates a complex communication cascade that results in a calcium spike in the root hairs. As a result, the plant produces what are known as infection threads, which are tubular structures that allow rhizobia to penetrate root hairs and get entangled in the curling roots. The formed nodule tissue is subsequently invaded by the infection threads. In the end, the peribacteroid membrane (PBM) that surrounds the invasive bacteria is produced via a form of endocytosis that takes place inside the plant cell. The bacteria develop into bacteroids that can convert air nitrogen to ammonium, and when plant and bacterial metabolism increase, the resulting symbiosomes flood the cytoplasm of the plant cells (Garg and Geetanjali, 2009).

3.1. Nodulation in Legumes

Plants in the Leguminosae family can form a nitrogen-fixing symbiosis with Rhizobia (Boivin, 1997). Rhizobia are soil bacteria that are members of the Rhizobium, Azorhizobium, and Bradyrhizobium genera. Both of them are capable of puncturing legume roots and causing morphological responses that lead to the formation of nodules (Pueppke, 1996). Rhizobia govern legume nodulation by means of a set of bacterial nodulation (nod) genes that are involved in the production of lipo-chitooligosaccharides (Nod factors), which serve as signaling molecules for nodulating certain legume hosts. The nodABC genes are found in all rhizobia because they are responsible for synthesising the basic building blocks of the Nod factors (Moulin et al., 2001).

Sensitive root hairs are seen in the Zone of Nodulation (ZON) at the time of inoculation; Autoregulatāon of Nodulatāon (AON) produces a nodulation phenotype in which most nodules form near the root system's crown. Nodulation has a pattern to it. Some ideas propose that AON inhibits cortical cell divisions by slowing them down in order to prevent nodulation from happening early in the division process. Figure 3 shows the nodulation stages (Reid et al., 2011).

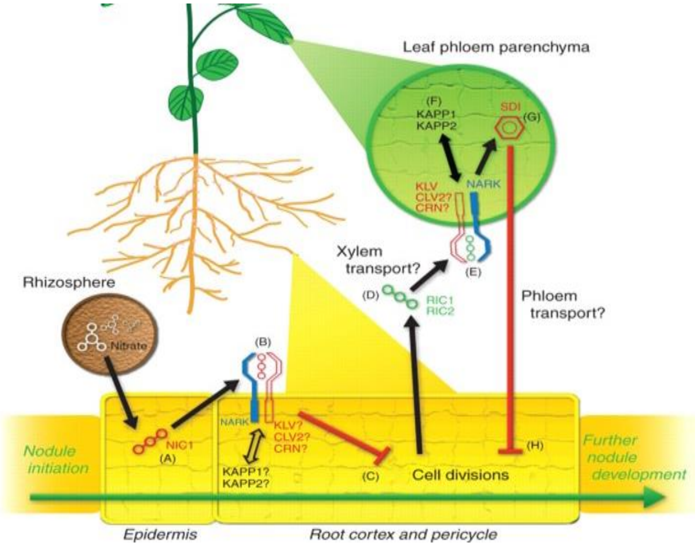


Figure 3. A working model of root and shoot mechanisms in autoregulation of nodulation (AON) (Reid et al., 2011).

As seen in Figure 3, legumes control nodulation in response to soil nitrogen levels and pre-existing illnesses. Nitrate in the root activates NARK (B), the AON receptor kinase, or the animal orthologues of these enzymes. Consequently, the production of a nitrate-induced CLE peptide (NIC1; A) occurs, which limits nodule development locally (C). NARK may be able to identify NIC1 through collaboration with other elements. Rhizobia-induced CLE peptides (RICs) can be transferred throughout the nodule's growth via xylem (D). NARK, maybe CLV2, KLV, and CRN (E) are required for the shoot to recognize these potential ligands. Following the phosphorylation of two kinase-associated protein phosphatases (KAPP1/2) by NARK, NARK kinase (F) is dephosphorylated. It's probable that the production of the shoot-derived inhibitor (SDI; G) requires phosphorylation balance between these components. The phloem carries SDI to the roots, where it inhibits further nodule development and cell division (H). It's probable that the nitrate route, which prevents nodule growth locally, also involves a substance that is comparable to SDI (Reid et al., 2011).

Nodulation is initiated by a signal exchange between the symbiotic partners. Rhizobia starts the process by identifying exudates from plant roots, mostly flavonoids. These molecules then activate the nodulation (nod) genes, which cause lipo-chitooligosaccharidic Nod factors (NFs) to be produced and released. When NFs are found in the root epidermis, LysM-type receptor-like kinases (RLKs) in the epidermis initiate a signaling cascade that promotes infection and cortical cell proliferation. Both nodule initiation and NF production are heavily reliant on the exacting structural specifications of both signal molecules. The majority of examined legumes, including the model legumes, have root hairs that allow bacteria to enter. The root hairs react to the NFs by influxing Ca_2+ at their tip and then effluxing Cl– and K+ right away. In order to capture a bacterial colony, these ion fluxes cause the root hairs, also known as shepherd's crooks, to distort and curl. The only root hairs that are sensitive to NF perception are the young, growing ones in a small area directly above the root tip. The tubular structures called intracellular infection threads, which are formed from plants and guide rhizobia into the underlying cortical cell layers, are characterized by localized hydrolysis and root hair cell plasma membrane invagination. Before the incoming threads, cortical cells dedifferentiate, divide, and create a nodular primordium. Decisive or indeterminate nodules are produced by nodules that emerge from the inner or outer root cortex, depending on the legume host. An infection zone containing cells with proliferating infection threads that discharge bacteria into plant cells' cytoplasm is found after the meristem. Symbiosomes, which resemble organelles, are created when the bacteria are absorbed and become enclosed in a plant-derived peribacteroid membrane. Within the fixation zone, the bacteroids that fix nitrogen are developed from the bacteria in the symbiosomes. cells (Mortier, 2012).

Different plant species may have different nodule sizes and shapes. Large, rounded nodules are typically seen on clover plants (*Trifolium* spp.) soybeans (*Glycine max*), faba beans (*Vicia faba*) and Alfalfa (*Medicago*) have smaller, more elongated nodules. Certain plant species possess nodules that are more asymmetrically formed. Leghaemoglobin is a pigmented protein found in actively N-fixing nodules. The presence of it causes the inside of nodules to become red, signifying the presence of living, active bacteria. The majority of dead, inactive, and selenium nodules have a brown or greyish-green color. Four to six weeks after seeding, nodules start to show signs and peak in activity around the time of flowering. At plant maturity, which occurs after blooming, the roots and nodules are senescent in the fall and some have begun to decompose. Nitrogen fixation may continue all season long after the perennial crop, such as clover ley, is harvested. In April, perennial legumes begin a new round of nodulation. (Pommeresche and Hansen 2017).

Figure 4. Active and non active nodules (Pommeresche and Hansen 2017).

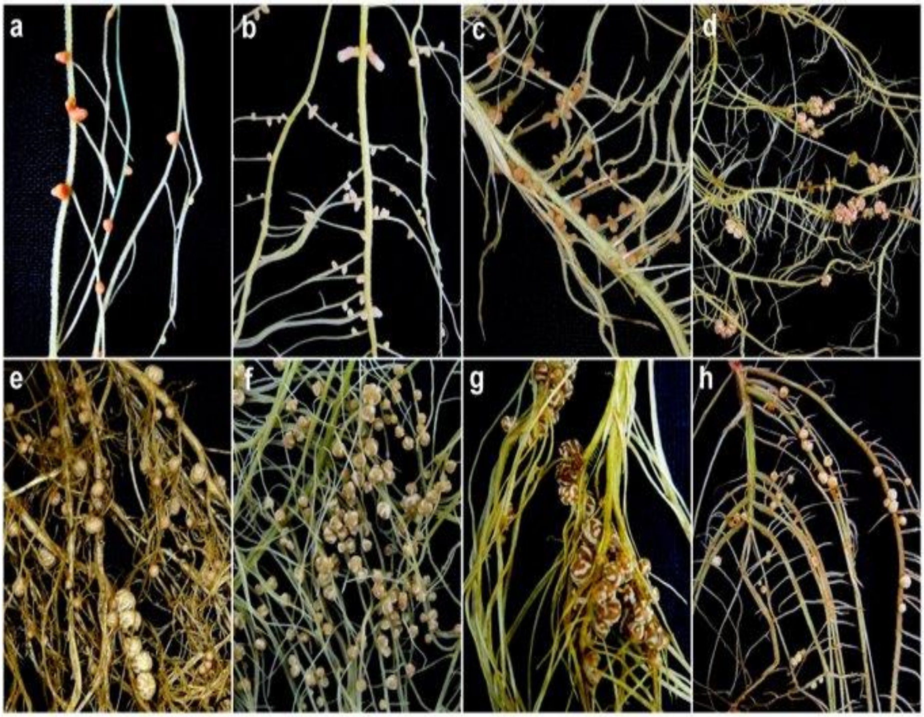
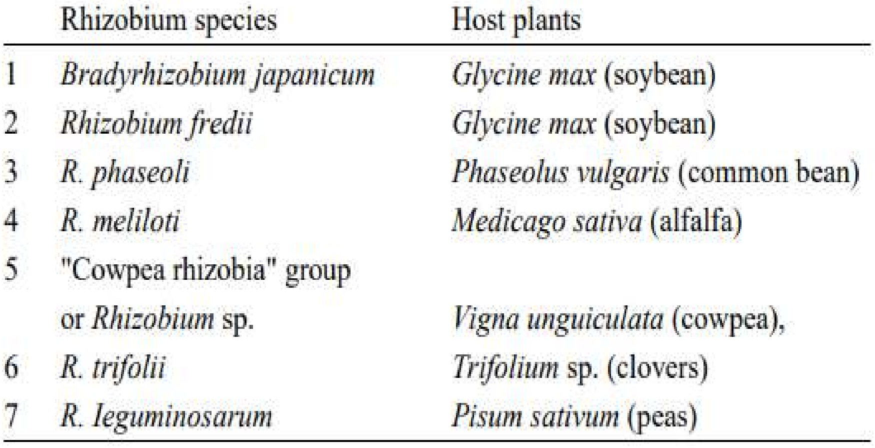


Figure 5. Diversity of legume root nodules (Foto by. Malina T.); (a) Lentil, (b) Alfalfa, (c) Pea, (d) Common bean, € Soybean, (f) Kidney bean, (g) Cowpea (h) Pigeon pea

Table 4. List of a Few Rhizobium species and their corresponding hosts (Dashora, 2011).



	Rhizobium species	Host plants
1	*Bradyrhizobium japanicum*	*Glycine max* (soybean)
2	*Rhizobium fredii*	*Glycine max* (soybean)
3	*R. phaseoli*	*Phaseolus vulgaris* (common bean)
4	*R. meliloti*	*Medicago sativa* (alfalfa)
5	"Cowpea rhizobia" group or *Rhizobium* sp.	*Vigna unguiculata* (cowpea),
6	*R. trifolii*	*Trifolium* sp. (clovers)
7	*R. leguminosarum*	*Pisum sativum* (peas)

3.2. Nitrogen Fixation in Legumes

Rhizobia, the symbiotic microbes found in root nodules, have the ability to absorb gaseous nitrogen (N_2) from the atmosphere and "fix" it into molecules that the plant can absorb, such as amino acids or ammonia. Dicarboxylic acids, a source of carbon, are given to the rhizobia by the plant as payment (Soussi et al., 1999). The nitrogen-fixing enzyme, nitrogenase, is irreversibly destroyed when it comes into contact with oxygen. Leghemoglobin, a protein that resembles human hemoglobin, is produced by the plant in order to provide oxygen to the rhizobia in the nodules. This protein often lends pink color to functional nodules. (Bruning and Rozema, 2013). Because of this symbiotic relationship, a variety of regulatory systems have developed specifically to maximize a plant's N-demand in combination with its nodule activity. Among these procedures are the following ones: Reactive nitrogen species (RNS) and reactive oxygen species (ROS) are produced; leghemoglobin (Lb) regulates the supply of oxygen to nodules and limits O_2 diffusion through a physical barrier called the oxygen diffusion barrier (ODB); molecular control is achieved by adjusting the number of nodules and N_2 activity. Complex regulatory mechanisms, such as those that govern the network of expressed genes and the nutrient-dependent cellular metabolism in the bean plant's shoot through sensing and long-range signaling cross section, underpin these activities. (Schwember et al., 2019).

In terms of chemistry, BNF is basically the nitrogenase-catalyzed conversion of dinitrogen (N_2) to ammonia.

The catalyzed reaction can be shown as follows:

$$N_2 + 8\,H^+ + 8\,e^- \xrightarrow{16Mg\ ATP \;\to\; 16Mg\ ADP + 16Pi} 2NH_3 + H_2\uparrow$$

Although the N_2 fixation process is undoubtedly far more complicated than this, the biological reaction identifies the essential components and highlights the crucial conditions. It shows the dual demand for lowering potential as well as the significant energy requirements for ATP. It also implies that a method of using ammonia is required in order to neutralize the alkalinity produced at the same time (Saikia and Jain, 2007).

Nitrogenase, the enzyme in charge of fixing nitrogen dioxide, is exclusive to prokaryotes. It is made up of two metalloproteins that are permanently damaged when they come into contact with oxygen. After being activated by MgATP, the Fe-protein provides electrons, and the MoFe-protein binds N_2. Nitrogenase can reduce substrates including acetylene, azides, and cyanides, and this capacity has been used to gain vital information. H_2 is generated during the reduction of N_2 to NH_3, and it can become the only reaction in the absence of N_2. MgATP in sufficient amounts are necessary for all of these processes. One major physiological restriction on the usage and distribution of nitrogenase is its sensitivity to oxygen; other significant restrictions include the presence of metals and the need for ATP. Nitrogenase production and occasionally its function is regulated by O_2 and NH_3 (Postgate, 1982).

The process of atmospheric dinitrogen conversion to ammonia, which is necessary for biological nitrogen fixation, is catalyzed by the nitrogenase enzyme system and requires ATP. The two constituent metalloproteins that make up nitrogenase are the MoFe-protein, which associates electron transfer with ATP hydrolysis by using the FeMo-cofactor as its active site, and the Fe-protein. The fundamental mechanism of nitrogenase is as follows: Until enough protons and electrons are collected to allow the reduction of accessible substrates, this cycle is repeated. (i) The MoFe-protein and two bound ATP combine with the reduced Fe-protein to create a complex. (ii) The hydrolysis of ATP is connected to the transfer of electrons between the two proteins. (iii) The Fe-protein dissociates and swaps ATP for ADP in tandem with re-reduction (facilitated by ferredoxins or flavodoxins). Nitrogenase is a catalytic enzyme that not only reduces protons to dihydrogen but also to non-physiological substrates like acetylene, which are tiny molecules that often include unsaturated bonds. The total reaction stoichiometry of the process catalyzed by nitrogenase is currently unclear. These uncertainties might be represented by the following equation (Figure 6) (Rees et al., 2005).

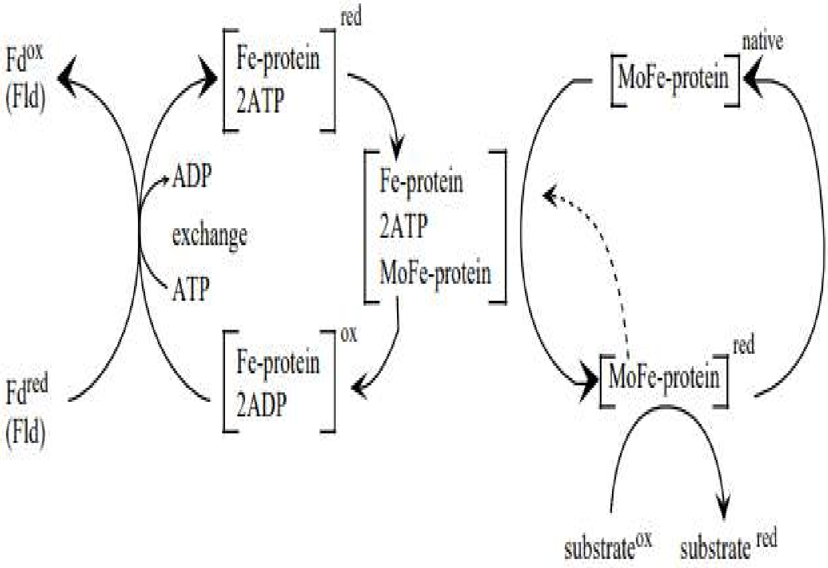


Figure 6. Schematic of the nitrogenase turnover cycle (Rees et al., 2005)

The diagram shows the following steps of the nitrogenase turnover cycle: Reduced substrates occur after the MoFe-protein returns to the resting redox state (right), electrons move from electron carriers like ferredoxin (Fd) or flavodoxin (Fld) to the Fe-protein (left), and electrons move from the Fe-protein to the MoFe-protein in relation to ATP hydrolysis (center).

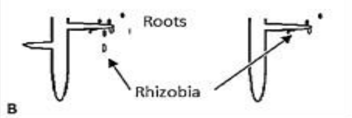
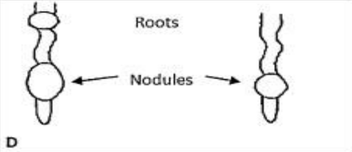
4. EFFECT OF SOME ENVIRONMENT FACTORS ON NITROGEN FIXATION IN LEGUMES

Every known habitat exhibits the phenomena of biological nitrogen fixing. The Rhizobium strain, the host plant's genotype, and the interactions between the symbionts and the pedoclimatic variables and ambient circumstances all have an impact on symbiotic nitrogen fixation. Because they stop rhizobia from colonizing the soil and the rhizosphere of legumes, pH extremes have an impact on nodulation. Nodulation is more impacted by extremely acidic soils (pH<4.0) than by host-plant development and nitrogen fixation; these soils also typically contain low levels of calcium, phosphorus, and molybdenum, as well as significant amounts of aluminum and manganese, which can occasionally be toxic to both partners. High levels of sodium chloride, bicarbonate, and borate are commonly found in highly salinized, very alkaline (pH>8.0) soils, which inhibit nitrogen fixation. Although nodulation and N-fixation can occur at a wide range of temperatures, the optimal range is between 20 and 30°C. In temperate legumes, elevated temperatures can impact the form and function of nodules, as well as postpone their beginning and development. However, the efficacy of nitrogen fixation is the primary influence on tropical legumes (Bordeleau and Prévost, 1994).

Lower root temperatures, under ideal circumstances, are more successful in preventing root hair infection than they are at promoting nodule initiation, nodule growth (including the formation and degeneration of bacteroid tissue), or nitrogen absorption. Warm temperatures at the roots speed up the breakdown of bacteroid tissue and prevent it from growing. Compared to temperate species, tropical and subtropical legumes require greater minimum temperatures for the development of nodules. Nodulation and nitrogen fixation are affected by both high and low shoot temperatures, albeit not to the same extent as they would be at equivalent root temperatures (Gibson, 1971).

Rhizobia population declines during the dry season are probably the cause of poor legume nodulation in desert soils. Consequently, fixation also tends to decline as legumes mature, mostly because to the concurrent rise in soil N lack of calcium, whether or whether low pH is a complicating factor, also influences rhizobia's adherence to root hairs. It's possible that rhizobia can withstand differing levels of soil acidity than the host plant. On the other hand, it has also been demonstrated that high root temperatures affect bean development, N_2-fixation capacity, and infection (Mohammadi et al., 2012).

In salty environments, the majority of legumes exhibit stunted development, fewer root hairs, and more frequently malformed root hairs. Since nodule formation depends on normal root hair growth, the initial inhibitory effect of salt is precisely this. Successful nodulation also requires a large population of rhizobia, and there is evidence that rhizobia count decrease in salinity. Figure 7 illustrates how various legumes react to salt (Bruning and Rozema, 2013).

Schematic drawing Non-saline Saline	Phase in nodulation	Problems under salinity (references)
	**Roothair formation**	↓plant growth ↓number of root hairs (2) Roothairs deformed (2, 7)
	**Communication, Rhizobia growth and physical contact**	↓ possibilities for signal exchange between partners(2) ↓ growth of Rhizobia (7, but 6 no effect)
	**Roothair curling and infection thread formation**	↓curling (2, 5, 7) ↓root hair expansion (5) ↓infection thread formation (2) bacterial release from infection thread into cells disturbed
	**Nodule formation and functioning (i.e. nitrogen fixation)**	↓nodule number (1, 4, 5, 6, 7, 9). Nodule morphology altered; necrosis in nodule (↑ nodule weight (1, 4, 6) ↓ nodule weight (4, 8, 9) ↑sugars and proteins in nodules (1, 8) ↓Leghemoglobin (Lb) content (8; high salt concentrations) ↓Acetylene reduction activity (1, 6, 8)

In greenhouse trials, N fertilization decreased BNF by around 70% compared to the unfertilized control, whereas in field experiments, it decreased BNF by approximately 44%. Applications for the vegetative stage had a greater impact than those for the reproductive stage. In comparison to the unfertilized treatments, BNF was boosted during fertilization with more nutrients. Compared to the unstressed control, water stress decreased BNF by 40%. When water stress was given during the vegetative (-70%) as opposed to the reproductive (-30%) stages, the detrimental effects were greater. As applied during the vegetative stage, flooding had the greatest effect, reducing BNF by 40% as compared to the non-flooded control (-82%). BNF nitrogenase activity decreased with increasing temperature. Enhancement with carbon dioxide has a stimulating impact on BNF. Soybean and Rhizobium fitness demonstrated a strong positive association across nutrients and environmental conditions, with the exception of N fertilization (Kirova and Kocheva, 2021).

Nitrogen is the most important mineral fertilizer for crop yield, followed by phosphorus (P). Poorly soluble mineral phosphates make up a significant portion of soil P at any given moment. For nodulation, a strong phosphorus supply is required. Legumes that rely on symbiotic nitrogen may therefore experience a nitrogen shortage if they do not get enough phosphorus. Rhizobia need certain proteins for N_2 fixation, and copper is one of those proteins. In underground clover, a Cu deficit reduced nitrogen fixation. A number of important nitrogenase complex enzymes, the electron transporter ferredoxin, and some hydrogenases all require iron. Legumes have an especially high iron demand because of the heme component of hemoglobin. Since molybdenum is an essential element for nitrogenase, a high molybdenum requirement applies to all N_2-fixing methods. Due to molybdenum insufficiency, legumes that rely on N_2 fixation often have nitrogen shortfalls. This is especially true in acid mineral soils that are common in humid and subhumid tropical regions. The discovery that a nickel-dependent hydrogenase is active in a variety of rhizobial bacteria has verified the unique function of nickel in nitrogenfixing bacteria. Cobalt is a crucial mineral component needed for the production of leghemoglobin and, consequently, for the development of legumes, which fix nitrogen in a symbiotic manner. It is well known that Rhizobium and other N_2-fixing bacteria, whether or not they grow inside nodules and whether or not they get their nitrogen from mineral nitrogen or N_2 fixation, always need cobalt (Weisany and Allahverdipoor, 2013).

REFERENCES

Agudelo, M. G., Ruiz, B., Capela, D., Remigi, P. (2023). The role of microbial interactions on rhizobial fitness. Frontiers in Plant Science, 14.

Allam, M.K.R. (2023). Investigating NodGRPs, GOLVEN10 Functions in Legume-Rhizobia Symbiosis and Root Architecture Control (Doctoral dissertation, Tennessee State University).

Berrada, H., Fikri-Benbrahim, K. (2014). Taxonomy of the rhizobia: current perspectives. British Microbiology Research Journal, 4(6), 616.

Bisset, K.A. (1952). Complete and reduced life cycles in Rhizobium. Microbiology, 7(3-4), 233-242.

Boivin, C., Ndoye, I., Molouba, F., de Lajudie, P., Dupuy, N., Dreyfus, B., de Bruijn, F.J. (1997). Stem nodulation in legumes: diversity, mechanisms, and unusual characteristics. Critical Reviews in Plant Sciences, 16(1), 1-30.

Bordeleau, L. M., Prévost, D. (1994). Nodulation and nitrogen fixation in extreme environments. Plant and soil, 161, 115-125.

Bruning, B., Rozema, J. (2013). Symbiotic nitrogen fixation in legumes: perspectives for saline agriculture. Environmental and Experimental Botany, 92, 134-143.

Dashora, K. (2011). Nitrogen yielding plants: the pioneers of agriculture with a multipurpose. American-Eurasian Journal of Agronomy, 4(2), 34-37.

Debellé, F., Moulin, L., Mangin, B., Dénarié, J., Boivin, C. (2001). Nod genes and Nod signals and the evolution of the Rhizobium legume symbiosis. Acta Biochimica Polonica, 48(2), 359-365.

Denison, R. F., Kiers, E.T. (2011). Life histories of symbiotic rhizobia and mycorrhizal fungi. Current Biology, 21(18), R775-R785.

De Ron, A.M. (Ed.). (2015). Grain legumes (Vol. 10). Springer.

Dwivedi, S.L., Sahrawat, K.L., Upadhyaya, H.D., Mengoni, A., Galardini, M., Bazzicalupo, M., Ortiz, R. (2015). Advances in host plant and rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes. Advances in agronomy, 129, 1-116.

FAO, 2023. https://www.fao.org/faostat/en/#data/QCL. Date of Access:15.12.2023

Garg, N., Geetanjali. (2009). Symbiotic nitrogen fixation in legume nodules: process and signaling: a review. Sustainable agriculture, 519-531.

Graham, P. H., Vance, C.P. (2003). Legumes: importance and constraints to greater use. Plant physiology, 131(3), 872-877.

Gibson, A.H. (1971). Factors in the physical and biological environment affecting nodulation and nitrogen fixation by legumes. Plant and Soil, 35, 139-152.

Ferraz Helene, L.C., Klepa, M. S., Hungria, M. (2022). New insights into the taxonomy of bacteria in the genomic era and a case study with rhizobia. International Journal of Microbiology, 2022.

Fischer, H.M. (1994). Genetic regulation of nitrogen fixation in rhizobia. Microbiological reviews, 58(3), 352-386.

Kirova, E., Kocheva, K. (2021). Physiological effects of salinity on nitrogen fixation in legumes–a review. Journal of Plant Nutrition, 44(17), 2653-2662.

Kuykendall, L.D., Young, J.M., Martínez-Romero, E., Kerr, A., Sawada, H. (2015). Rhizobium. Bergey's Manual of Systematics of Archaea and Bacteria, 1-36.

Lewis, I.M. (1938). Cell inclusions and the life cycle of Rhizobia. Journal of Bacteriology, 35(6), 573-587.

Lindström, K., Mousavi, S.A. (2020). Effectiveness of nitrogen fixation in rhizobia. Microbial biotechnology, 13(5), 1314-1335.

Martín-Cabrejas, M.A. (2019). Legumes: an overview. Legumes: Nutritional Quality, Processing and Potential Health Benefits, 1-18.

Mohammadi, K., Sohrabi, Y., Heidari, G., Khalesro, S., Majidi, M. (2012). Effective factors on biological nitrogen fixation. African Journal of Agricultural Research, 7(12), 1782-1788.

Mortier, V., Holsters, M., Goormachtig, S. (2012). Never too many? How legumes control nodule numbers. Plant, cell & environment, 35(2), 245-258.

Moulin, L., Munive, A., Dreyfus, B., Boivin-Masson, C. (2001). Nodulation of legumes by members of the β-subclass of Proteobacteria. Nature, 411(6840), 948-950.

Ormeno-Orrillo, E., Servín-Garcidueñas, L.E., Rogel, M.A., González, V., Peralta, H., Mora, J., Martínez-Romero, E. (2015). Taxonomy of rhizobia and agrobacteria from the Rhizobiaceae family in light of genomics. Systematic and applied microbiology, 38(4), 287-291.

Peter, J., Young, W., Haukka, K.E. (1996). Diversity and phylogeny of rhizobia. New phytologist, 133(1), 87-94.

Pommeresche, R., Hansen, S. (2017). Examining root nodule activity on legumes.

Postgate, J.R. (1982). Biological nitrogen fixation: fundamentals. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 296(1082), 375-385.

Pueppke, S.G. (1996). The genetic and biochemical basis for nodulation of legumes by rhizobia. Critical reviews in biotechnology, 16(1), 1-51.

Rees, D. C., Akif Tezcan, F., Haynes, C.A., Walton, M.Y., Andrade, S., Einsle, O., Howard, J.B. (2005). Structural basis of biological nitrogen fixation. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 363(1829), 971-984.

Reid, D.E., Ferguson, B.J., Hayashi, S., Lin, Y.H., Gresshoff, P.M. (2011). Molecular mechanisms controlling legume autoregulation of nodulation. Annals of botany, 108(5), 789-795.

Saikia, S. P., Jain, V. (2007). Biological nitrogen fixation with non-legumes: An achievable target or a dogma?. Current science, 317-322.

Schwember, A.R., Schulze, J., Del Pozo, A., Cabeza, R.A. (2019). Regulation of symbiotic nitrogen fixation in legume root nodules. Plants, 8(9), 333.

Sessitsch, A., Howieson, J.G., Perret, X., Antoun, H., Martínez-Romero, E. (2002). Advances in Rhizobium research. Critical Reviews in Plant Sciences, 21(4), 323-378.

Sinclair, T. R., Vadez, V. (2012). The future of grain legumes in cropping systems. Crop and Pasture Science, 63(6), 501-512.

Somasegaran, P., Hoben, H.J. (2012). Handbook for rhizobia: methods in legume-Rhizobium technology. Springer Science & Business Media.

Tharanathan, R. N., Mahadevamma, S. (2003). Grain legumes—a boon to human nutrition. Trends in Food Science & Technology, 14(12), 507-518.

Van Kessel, C., Hartley, C. (2000). Agricultural management of grain legumes: has it led to an increase in nitrogen fixation?.Field Crops Research, 65(2-3), 165-181.

Weisany, W., Raei, Y., Allahverdipoor, K.H. (2013). Role of some of mineral nutrients in biological nitrogen fixation. Bulletin of Environment, Pharmacology and Life Sciences, 2(4), 77-84.

Willems, A. (2006). The taxonomy of rhizobia: an overview. Plant and soil, 287, 3-14.

Yousuf, P. Y., Shabir, P. A., Hakeem, K.R. (Eds.). (2022). Advances in Plant Nitrogen Metabolism. CRC Press.

CHAPTER 9

LEGUME (*LEGUMINOSAE)* – MICROORGANISM (*RHIZOBIUM*) RELATIONSHIPS

Aykut ŞENER[1]

DOI: https://dx.doi.org/10.5281/zenodo.10450482

---

[1] Department of Field Crops,, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Turkiye aykutsener@isparta.edu.tr Orcid ID 0000-0003-1868-9451

INTRODUCTION

A significant part of plant-based proteins is provided by legumes. Edible legumes belonging to the *Leguminosae* family have an important place in human nutrition due to their high protein content (18-36%) and amino acids. Edible legumes; They are valuable plants in many aspects such as animal feeding, crop rotation, narrowing of fallow areas, nitrogen fixation to the soil. The nitrogen fixation in the soil by legumes is accomplished through rhizobium bacteria, which are capable of forming a symbiotic relationship with leguminous plants. These microorganisms infect the roots of leguminous plants, enabling them to fix atmospheric nitrogen into the soil. This symbiotic relationship between legumes and rhizobium, based on mutual benefit, is essential for humanity in many respects. This nitrogen, which is biologically fixed to the soil, is one of the most important nutrients necessary for plants. During biological nitrogen fixation, there is no environmental pollution, and no consumption of fossil-derived energy occurs. For these reasons, biological nitrogen fixation is one of the significant issues to be addressed for a sustainable world. To better understand symbiotic nitrogen fixation, it is necessary to have a good knowledge of both legumes and *rhizobium* bacteria, as well as the physiology and chemistry of symbiotic life. This section explains the relationship between legumes and rhizobium bacteria.

1. NITROGEN AND NITROGEN FIXATION

After carbon, hydrogen and oxygen, nitrogen is the most abundant element in plants. Nitrogen, which is high in the atmosphere (78%), is among the nutrients most needed by plants. Nitrogen, which is the building block of protein in plants; is found in the structure of chlorophyll, enzymes and vitamins. However, plants cannot directly utilize nitrogen from the atmosphere. In order for living things to use nitrogen gas (N_2) in the atmosphere, the triple bond between nitrogen molecules must first be broken. Reducing this triple bond to form a double bond and combining nitrogen with hydrogen and oxygen are essential steps. This process is called "nitrogen fixation". Nitrogen fixation in the soil occurs in three ways: industrial, atmospheric, and biological. Higher organisms, including plants, are autotrophic organisms capable of synthesizing their organic compounds from inorganic nutrients obtained from their environment. The assimilation of certain nutrients such as nitrogen and sulfur requires complex biochemical reaction series that demand the most energy in living organisms. The fixation (binding) of atmospheric nitrogen gas (N_2) by microorganisms is referred to as biological nitrogen fixation. It is reported that 175 million tons of nitrogen are fixed annually by biological nitrogen fixation (Sarıoğlu et al., 1993).

Non-Symbiotic Nitrogen Fixation

Only certain bacteria, blue-green algae, and fungi can directly benefit from atmospheric nitrogen. Bacteria capable of using atmospheric nitrogen directly are *Rhizobium, Clostridium, Azotobacter, Klebsiella, Bacillus, Amylobacter*, blue-green algae are *Anabaena, Nostoc, Calothrix, Oscillatoria* and fungi are *Mycorrhiza*. The fixation of nitrogen in this way is called "non-symbiotic nitrogen fixation". With free-living bacteria, 10-40 kg/ha of nitrogen can be bound from the atmosphere every year and converted into a form useful to plants (Brohi et al., 1997).

Symbiotic Nitrogen Fixation

If nitrogen fixation to the soil is done with symbiotic living bacteria, this is called "symbiotic nitrogen fixation". With symbiosis, nitrogen is provided to the host plant by the microorganism, while nutrients (carbohydrates) are provided to the microorganisms by the host plant. Among the known symbiotic microorganisms are *Actinomycetes* and *Rhizobiums*. Among these microorganisms, *rhizobium* bacteria select their host plants and form a symbiotic life with plants belonging to the *leguminosae family*. They perform symbiotic nitrogen fixation by forming nodulation in the roots of legume plants (Uyanık et al., 2011). Another common type of symbiosis is between soil bacteria of the genus *frankia and a few woody plant species known as actinorhizal plants, such as alder. Other types of symbiosis involving nitrogen fixation include the associations between the Gunnera and the cyanobacteria Nostoc and Anabaena with the aquatic fern Azolla.* In agriculture, *rhizobium* bacteria that form symbiotic relationships with leguminous plants are the most important nitrogen (N_2) fixers. Therefore, the relationships between legumes and *rhizobium* will be detailed in the following section.

2. LEGUMES (*LEGUMINOSAE*)

The legume (*leguminosae*) family is a large family that typically includes herbaceous plants, along with shrub and tree species. There are about 700 genera and 18000 species. Shrubs, annual herbaceous plants, and perennial tree forms have spread worldwide. (Şener et al., 2022). Legumes are used in many areas such as human and animal nutrition, pharmaceutical industry, cosmetics, dyeing and gum industry. In addition to wide distribution of legumes worldwide, 150 legume species are of great economic importance (Adak, 2021). Broad beans (*Vicia faba L.*), peas (*Pisum sativum L.),* cowpea (*Vigna sinensis* L.), beans (*Phaseolus vulgaris* L.), lentils (*Lens culunaris* Medik.) and chickpeas (*Cicer arietinum* L.) are used as edible legumes among legume species (Akçin 1988).

In 2020, the cultivation area of edible legumes worldwide was 79 million 572 thousand hectares, with a production quantity of 78 million 381 thousand tons (Kadakoğlu and Karlı, 2022). Edible legumes, which are produced in very large areas, are very important in terms of human nutrition, animal nutrition and crop rotation. In the world and in Turkey, edible legumes have an important place in human nutrition for thousands of years as the main source of plant-based protein (Akova, 2009). Dry grains of edible legumes contain high-quality plant-based protein (18-36%), which is twice as high as cereal grains. Proteins in edible legumes are poor in methionine amino acid and very rich in lysine amino acid. The proteins in edible legumes are considered equivalent to animal protein in terms of lysine, an essential amino acid found in very low levels in cereals (Pekşen and Çok, 2005). The degree of digestibility of plant-based proteins in edible legumes is quite high. Additionally, they are rich in vitamins A, B, C, and D, as well as minerals such as calcium (Ca), iron (Fe), potassium (K), and phosphorus (P). Due to their high nutritional value, edible legumes are crucial for human nutrition.

Edible legumes live a symbiotic life with *rhizobium* bacteria, fixing the free nitrogen of the atmosphere. This nitrogen, which is fixed in a symbiotic way, binds to the soil and the soil is enriched with nitrogen. Through symbiotic nitrogen fixation, legumes provide approximately 70% of the nitrogen they need. Although it varies according to the varieties and growing conditions; edible legumes fix nitrogen to the soil in the range of 5-20 kg/da annually (Önder, 2014).

3. *RHIZOBIUM*

Rhizobium is a genus of bacteria belonging to the *rhizobiaceae* family. *Rhizobiums* are aerobic, gram-negative, and rod-shaped bacteria. In 1888, Beijerinck isolated organisms responsible for N_2 fixation in leguminous plants and named them *Bacillus radicicola*. This name was later changed to *"rhizobium"* (İsmaillebioğlu, 1980; Paul and Clark, 1989). These bacteria colonize the roots of plants by entering them and form colonies in the shape of root nodules. Through nodules, atmospheric nitrogen gas (N_2) is converted to ammonia (NH_3). This formed ammonia provides the plant with organic compounds (glutamine and ureides). In return, the plant supplies the bacteria with organic compounds resulting from photosynthesis (Sawada et al., 2003). There are differences between the morphology and physiology of *rhizobium* in its free-living state and in the bacteroid state within nodules. The *rhizobium* genus includes the genera *Allorhizobium* sp., *Azorhizobium* sp., *Bradyrhizobium* sp., *Mesorhizobium* sp., and *Sinorhizobium* sp. (Vance, 2001).

4. LEGUME – *RHIZOBIUM* RELATIONSHIPS

The ability of a specific rhizobium species isolated from the nodule of any legume plant to form nodules in other legume plants from which it was isolated is called "cross-inoculation." The legume species and cross-inoculation groups are shown in Table 4.1.

Table 4. *Rhizobium* cross-inoculation strains

Genus/type of bacteria		**Host plant**
Rhizobium leguminosarum	biovar. *viciae*	Vetch, lentils, peas
	biovar. *phaseoli*	Bean
	biovar. *trifolia*	Clover
Rhizobium meliloti		Alfalfa, melilot
Bradyrhizobium japonicum		Soybean
Bradyrhizobium lupinus		Lupine
Bradyrhizobium arachis		Peanut

Rhizobium bacteria exist freely in the soil. As a result of the signal sent by the host plant, the bacteria infect the host plant. In the period before the bacteria and the legume plant establish a symbiotic relationship, signals are sent from the root zone of the plant to stimulate the bacteria. The presence of specific genes is necessary for signal formation, the process of infection and the development of nodules, both in the host and in the bacteria. There are 17 nitrogen fixation genes in bacteria. These genes are known as "nod and nif" genes. While nodA, nodB, and nodC genes are present in all *rhizobium* strains, genes like nodD, nodE, nodL, nodP, nodQ, and nodH show variations among *rhizobium* species. Nif genes are responsible for the synthesis of the nitrogenase enzyme, the electron transport required for the binding of molybdenum and iron to the nitrogenase enzyme, the protection of nitrogenase from oxidation, and the regulation of the activity of nitrogenase according to the amount of nitrogen in the environment (Ahemad and Kibret, 2014). Biological nitrogen fixation is a process carried out through the enzyme nitrogenase (Santi et al., 2013). The nitrogenase enzyme consists of iron (Fe)-containing dinitrogenase reductase and molybdenum (Mo)-containing dinitrogenase. Dinitrogenase reductase is involved in electron transfer and utilizes the received electrons to reduce nitrogen gas (N_2) to ammonia (NH_3) (Santi et al., 2013).

The first stage in the formation of a symbiotic relationship is the movement of free-living bacteria in the soil towards plant roots. This action is triggered by chemical stimulants such as flavonoids and betaines released from plant roots. The bacterium, stimulated by the chemical released from the plant, activates the nodD gene. The nodD gene, in turn, activates other nod genes. As a result of the biosynthesis of host-specific node genes, legume-rhizobium pairing is completed. Tryptophan released from the roots of leguminous plants is converted to indoleacetic acid (IAA) by *rhizobium* bacteria. Through IAA, legume roots elongate, curl, and weaken the cell walls of the roots, facilitating the infection of the legume root by the bacterium (Oldroyd and Downie, 2008). *Rhizobium* enters the plant cell through the tip of the weakened root hair. The root hair cells of the plant curl abnormally and bacteria multiply in this fold. Subsequently, absorbent hairs form a thread-like structure thought to be continuous with cell wall components, known as the infection thread. This thread generally reaches the base of the absorbent hair cell 48 hours after infection (Paul and Clark, 1989). The multiplying bacteria spread from the growing tip of the infection thread to the host cell cytoplasm, where they take on the bacteroid form, and nitrogen fixation occurs in this form (Haktanır and Arcak, 1997; Kaçar and Katkat, 2006). At this stage, legume roots have budded, forming nodules. These nodules are pink due to a protein called leghemoglobin. Leghemoglobin, which is formed as a result of symbiotic life, is responsible for carrying oxygen to the nodules from the outside. In addition, leghemoglobin maintains the amount of oxygen at the level at which the enzyme nitrogenase can be active.

Nutrient Translocaiton and Nitrogen Fixation

A symbiotic life begins with the exchange of nitrogen fixed by bacteria and nutrients provided by the plant. Bacteria produce ammonium by reducing the free nitrogen of the air. In the formula given below, N_2 is reduced to ammonium through the enzyme nitrogenase. Bacteria provide the energy required for nitrogen fixation from plants. Sugars synthesized during photosynthesis in plant leaves are transported to the roots or used as electron donors for nitrogen fixation, either directly or by converting them into organic acids (Brock, 1974).

$$N2 + 8_{e}- + 8\ H+ + 16\ ATP \rightarrow 2\ NH3 + H_2 + 16\ ADP + 16\ Pi$$

To avoid the toxicity of ammonia released by symbiotic nitrogen-fixing prokaryotes, it must be rapidly converted to organic forms in root nodules before it can be transported to the stem via xylem. The mechanism known as the glutamine synthetase-glutamate synthase pathway is responsible for the entry of ammonia into organic structures (Marschner, 1995). In chloroplasts, glutamine synthetase transfers the newly formed NH_4^+ to glutamate by consuming ATP, and as a result of this transfer, glutamine is formed. Nitrogen is transported in amide or ureide form, depending on the composition of the xylem sap. In peas, chickpeas, and lentils, nitrogen is transported in the form of asparagine or glutamine (amide), while in plants like soybeans and beans, it is transported in the form of ureide (Marschner, 1995).

FACTORS AFFECTING NODULE FORMATION AND NITROGEN FIXATION

Microorganisms in the soil are greatly affected by environmental conditions Under ideal conditions, they proliferate rapidly, while in unfavorable conditions, they strive to survive. Nodule formation and nitrogen fixation are affected by many factors. Soil nutrient elements, chemicals, physical and biotic factors have effects on the survival of bacteria, the formation of nodules, the fixation of nitrogen and the development of the plant. Deficiency or excess of any nutrient element in the soil can directly or indirectly affect fixation. For example, a low amount of nitrogen in the soil promotes nodule formation, while high nitrogen doses negatively impact nodulation, reducing nitrogen fixation. Therefore, nitrogen fertilization is applied to leguminous plants in small amounts. With a small amount of nitrogen fertilizer, the plants are provided with the necessary nitrogen until the nodule is formed. Nitrogen in small amounts in the soil increases of nodule numbers and nitrogen fixation. Phosphorus is effective in accelerating the formation of nodules, increasing the size and number of nodules by increasing the root development of plants and the activity of *rhizobium* bacteria. Iron (Fe) and molybdenum (Mo) are particularly important for nitrogen fixation due to their presence in the structure of the nitrogenase enzyme. If molybdenum is not found in the environment, nodules may form, but nitrogen fixation cannot occur. For effective nitrogen fixation, it is necessary for all nutrient elements to be present in sufficient quantities in the environment.

Soil pH and temperature are also crucial for the free-living existence of *rhizobium* bacteria, their ability to form nodules, and nodular activity. *Rhizobium* bacteria need to have a soil pH between 4.6 and 8.5, and they reproduce best in soils with a pH of 6.8 (Singleton et al., 1982). In addition, Ca, Mg and Mo deficiencies can be seen in acidic soils. In acidic soils, Al and Mn have a toxic effect on the plant and root development is adversely affected. Like soil pH, soil temperature also affects microbial activity. Low and high temperatures have adverse effects on nodulation and fixation. In addition, soil moisture and soil aeration also affect fixation. *Rhizobium* bacteria are sensitive to both drought and excessive water. In cases where soil aeration is poor, nodule development weakens.

The compatibility of the bacterial strain and the legume plant also has an effect on nitrogen fixation. If legumes are to be grown in an area where legumes have not been cultivated before, it is necessary to inoculate with the suitable bacterial strain that specific to that plant species. There may be bacteria that live freely in the soil and have the ability to infect that plant. However, because the density of these bacteria in the soil and their exact compatibility with the plant are unknown, inoculation is the most accurate option. Although some *rhizobium* bacteria have the ability to infect multiple leguminous plant species, they may not exhibit the same nodulation and nitrogen fixation capabilities in every plant. For effective nitrogen fixation, it is crucial for root infection to be robust, for there to be compatibility between *rhizobium* and the host plant, and for the *rhizobium* bacteria to compete successfully with other microorganisms in the soil. In light of the information provided above, it can be said that the compatibility between legumes and rhizobium is significant.

CONCLUSION

The symbiotic relationship between legumes and *rhizobium* is not obligatory. Seeds of leguminous plants can germinate and develop into adult plants without any bacteria. The plant can sustain its life without *rhizobium*. *Rhizobium* bacteria also live freely in the soil. Symbiosis life begins with the exchange of signals of the *rhizobium* and the plant. In soil conditions where nitrogen is limited, symbionts find each other through signal exchange. The infection process following this signal formation and the development of nodules in which nitrogen is fixed require the presence of special genes in the symbionts. Thanks to these specific genes, a matching occurs between a suitable host for *rhizobium* and a suitable *rhizobium* bacterium for the host. Despite not being obligatory for each other, this symbiosis takes place. Through symbiotic life, atmospheric nitrogen is fixed in the soil and most of the nitrogen needed by the legume plant is met.

In 2020, legume cultivation was carried out on an area of 79 million 572 thousand hectares. Thanks to the legume-*rhizobium* symbiosis, an average of 5-20 kg of nitrogen per decare is fixed in the soil annually. Worldwide, approximately 4 million to 15.9 million tons of nitrogen are fixed in the soil annually through legume cultivation alone. The total industrial ammonia production required for nitrogen fertilizer production in the world is 114 million tons (Anonymous, 2018). Between 2.85-7.1% of the total industrial nitrogen produced in the world is fixed in the soil by legume agriculture. This amount is significantly important and should not be underestimated. To fix this amount of nitrogen into the soil industrially, a substantial amount of energy is required. As a result of the Covid19 pandemic and today's wars, we are facing an energy crisis. Since industrial nitrogen fixation is a completely energy-dependent sector, production has been disrupted. In addition to the problems in the fertilizer industry, the importance of nitrogen fixation in natural ways is once again emerging in this period when sustainable and ecological agriculture is on the agenda intensively.

In conclusion, the non-obligatory symbiotic relationship between legumes and rhizobium bacteria, along with its biology, physiology, and chemistry, has been briefly explained in this chapter. Furthermore, the economic and environmental significance of this symbiotic relationship has been elucidated. Substantial nitrogen acquisition can be achieved through symbiotic nitrogen fixation. Supporting the cultivation of leguminous plants in fallow areas will not only increase production but also introduce biologically fixed nitrogen into the soil. Additionally, inoculating the soil with rhizobium bacteria will enhance nitrogen fixation through the host plant. Considering that the atmosphere is the most significant nitrogen source in nature, fixing atmospheric nitrogen into the soil through symbiotic nitrogen fixation is the least harmful, economical, and easily achievable method. Humanity must benefit from this non-obligatory symbiotic relationship for a more sustainable world.

REFERENCES

Adak, M.S. (2021). *Yemeklik Baklagiller*. Ankara Üniv. Ziraat Fakültesi, Yayın No: 1652, Ders Kitabı: 603. 312 s., Ankara.

Ahemad, M. Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. Journal of King Saud University-Science, 26(1), 1-20.

Akova, Y. (2009). İGEME Bakliyat Raporu. T.C. Başbakanlık Dış Ticaret Müsteşarlığı İhracatı Geliştirme Etüd Merkezi, Ankara.

Anonim, (2018). Gübre Sektör Politika Belgesi 2018-2022. 15 Kasım 2021, Erişim adresi: https://www.tarimorman.gov.tr/TAGEM/Belgeler/yayin/G%C3%BCbre%20Sekt%C3%B6 r%20Politika%20Belgesi%202018-2022.pdf

Beijerinck, M.W. (1888). The root-nodule bacteria. Bot. Zeitung, 46, 725-804.

Brock, J. L. (1974). Effects of summer grazing management on the performance of ‘Grassland Huia’ and ‘Grassland 4700’ white clovers in pastures. N.Z. J. Exp. Agric. 2, 365–369.

Brohi, A. R., Aydeniz, A., Karaman, M.R. (1977). Toprak Verimliliği. Türk Hava Kurumu Basımevi, Ankara.

Haktanır, K. Arcak, S., (1997). Toprak Biyolojisi. Ankara Üniversitesi Yayın No: 1486. Ankara.

İsmailçelebioğlu, Y.N. (1980). *Rhizobium*'dan Agrobacterium'a Nif Genleri Transformasyonu Yoluyla Baklagil Olmayan Kültür Bitkilerinde Ürün Artırma Olanaklarının Araştırılması. Atatürk Üniversitesi Yayın No: 564. Ziraat Fakültesi Yayın No: 254. Araştırma Serisi No: 167. Erzurum.

Kacar, B., Katkat, V., Öztürk, Ş. (2006). Bitki Fizyolojisi. Nobel Yayınları, No: 848.

Kadakoğlu, B., Karlı, B. (2022). Türkiye’de Yemeklik Tane Baklagiller Üretimi, Tarım Politikaları ve Dış Ticaretinin Rekabet Gücü Analizi, Ziraat Fakültesi Dergisi, 17:75-87.

Marschner, H. (1995). Mineral nutrition of higher plants. 2nd Print, Academic Press. London.

Oldroyd, G. E., Downie, J. A. (2008). Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu. Rev. Plant Biol., 59, 519-546.

Önder, M. (2014). Türkiye'de Yemeklik Tane Baklagillerin Önemi ve Tarımı. Yemeklik BaklagilÇalıştayı / 05-06 Mart 2014. Konya

Paul, E.A., Clark, F.E. (1989) Soil Microbiology and Biochemistry. Academic Press, San Diego, 272p.

Pekşen, E., Artık, C. (2005). Antibesinsel maddeler ve yemeklik tane baklagillerin besleyici değerleri. *Anadolu Tarım Bilimleri Dergisi*, *20*(2), 110-120.

Santi, C., Bogusz, D., Franche, C. (2013). Biological nitrogen fixation in nonlegume plants. Annals of Botany, 111(5), 743-767.

Sarıoğlu, G., Özçelik, S., Kaymaz, S. (1993). Selection of effective nodosity bacteria (*Rhizobium leguminosarum* biovar. *viceae*) from lentil grown in Elazığ. Turkish Journal of Agriculture and Forestry, 17: 569-573.

Sawada, H., Kuykendall, L. D., Young, J. M. (2003). Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts. The Journal of general and applied microbiology, 49(3), 155-179.

Singleton, P.W., El Swaiyf, S.A., Bohloal, B.B. (1982). Effect of salinity on *Rhizobium* growth and survival. Applied and Environmental Microbiology, 44: 884-890.

Şener, A., Yılmaz, E.D., Kaya, M., Çiftçi C.Y. (2022). Türkiye'de Baklagil Aymazlığı. Ziraat, Orman ve Su Ürünleri Alanında Güncel Tartışmalar, 241-257.

Uyanık, M., Rezaeieh, K. A. P., Delen, Y., Gürbüz, B. (2011). Baklagillerde bakteri aşılaması ve azot fiksasyonu. Ziraat Mühendisliği, (357), 8-12.

Ülgen, Z. (1975). Baklagil Bitkilerinin Nodül Bakterileri İle Aşılanması. TC Köy İşleri Bakanlığı. Toprak Su Genel Müdürlüğü, (56).

CHAPTER 10

PRE AND AFTER DISEASE MANAGEMENT OVER INFECTIONS

Şerife Nergis BAYAR [1]

DOI: https://dx.doi.org/10.5281/zenodo.10450484

---

[1] Aegean Agricultural Research Institute, Plant Protection, Menemen/İzmir, Turkiye. nergiscelik1985@gmail.com, ORCID ID: 0000-0003-0488-4299

INTRODUCTION

Plant protection; these are the methods used to prevent and reduce the damage caused by disease factors like pests, weeds and other organisms that may cause damage to plants and their products in agricultural areas and to reduce their density below the economic damage level. For better quality and higher efficiency per unit area and increasing the economic income level is possible with plant protection (Lo Presti et al., 2015). Countries are came face to face with purchasing agricultural-based industrial products to meet their food necessity. Herbal products and various substances of plant origin move from country to country, so plants and herbal products cross national borders and spread all over the world in a very short time. Today, there is a fast and large scale plant exchange in the world. As a result, plant and plant product parts are spread to different places in a short time. Along with these dangerous diseases, pests and weeds are also spread around the World (Scharf et al., 2014; Ludwg Müller, 2015; Ryder and Talbot, 2015). If precautions are not enough, uninfected countries and regions become infected with harmful pests, diseases or wees. These factors, which used to take a long time to spread now occur in a short time (Lo Presti et al., 2015). Today, approximately 550 diseases, pests and weeds that cause economic damage in more than 100 cultivated agriproduct grown in our country. People do not harvest what they have planted, they harvest what is left over from diseases, pests and weeds. The main purpose of the plant protection should be to share these crop-reducing plant protection factors without damaging the natural balance (Kadıoğlu, 2012). Agricultural control; it covers all kinds of activities carried out to prevent the negative effects of diseases, pests and weeds that cause product losses in plants and products.

Agricultural control should be carried out in a way that does not harm human and environmental health and the natural balance. The aim of agricultural management is not to completely eliminate pests, but also to keep the density of pests below the level of economic damage (Anonymous, 2023a). If we talk about the classical control methods against plant protection factors, they can be grouped under the headings of cultural management, mechanical management, physical management, biological management and chemical management. Although some of them are within this scope, we can consider quarantine management, genetic and biotechnical methods and integrated combat as separate combat methods.

1. CULTURAL MANAGEMENT

1.1. Growing strong and healthy plants, which are selected plants less affected by pests.

1.2. Soil tillage is a cultural method that accelerates the development of the plant and ensures the development of a good root system and a strong one in order to grow strong and healthy plants.

1.3. Fertilization, balanced and good fertilization contributes to a better development of the plant. It is necessary to use many types of organic and chemical fertilizers used for this purpose on the appropriate plant at the appropriate time and field.

1.4. Infrequent cultivation is necessary for plants to be ventilated, exposed to light, to avoid pests and diseases, and to grow strongly.

1.5. Irrigation and drainage, the plant is affected by too much or less water. Some pests reproduce through water. Drainage is important in soils that retain a lot of water.

1.6. Using clean seeds, seedlings, saplings and other production materials are important in terms of diseases and pests. Plant seeds mixed with weed seeds causes unhealthy development. It is very important and necessary to use certified seeds in production.

1.7. Rejuvenation and pruning causes some plants to develop vigorously and be less affected by pests and diseases.

1.8. Useing sterile equipment, if the tools which are used in soil cultivation and pruning will be used elsewhere, they must be cleaned and disinfected. This prevents the pest and disease from being transferred to another plant.

1.9. Adjusting harvest time should not be done at a time that will cause excessive damage to the plant and cause product loss.

1.10. Crop rotation, continuously growing the same plants in the soil can cause the soil to become tired. In addition, growing the same plant continuously may increase the density of diseases and pests.

1.11. Destruction of plant residues and weeds is also very important. Disease infected branches must be colleted from fields for subsequent production.

2. PHYSICAL MANAGEMENT and MECHANICAL CONTROL

It is the application of radiation and heat through mechanical methods to remove diseases and pests and prevent their formation. Disinfecting the soil with the help of solar energy called solarization which reduces population of nematodes and pathogens in the soil. It can be lined up like; to collect, to obstruct catch with traps, solarization, immersion in water, mulching, burning and take advantage of the heat.

3. BIOLOGICAL CONTROL

Biological control is the use of other living oraganisims that will reduce the population of pests instead of chemicals. These are studies carried out to keep the pest population under the economic damage threshold by using living organisms against pests and weeds in cultivated plants. Biological control:

It protects the natural balance.

It has no negative effects on the environment and human health.

Management costs are reduced and economic savings are achieved.

A product without drug residue is obtained.

4. BIOTECHNICAL CONTROL

Disrupting the normal characteristics of the pest by using some artificial and natural substances that are effective on the pest's biology, physiology and behavior. The struggle using some natural or artificial substances that have an effect on the life and behavior of pests is called biotechnical control. For this purpose, pheromones, sticky traps, light and water traps are used. Light traps, some insects are affected and attached to light. So prepared light traps help to catch the insects that head there. Traps may change according to insects. Sticky traps, some pests such as aphids and whiteflies can be controlled with yellow sticky traps, and thrips can be controlled with blue sticky traps. Pheromone traps, pheromone is a chemical substance secreted by insects that attracts the opposite sex to the source of smell. For example, it is used to catch insects in forest plants.

5. CHEMICAL MANAGEMENT

It is the reduction or elimination of pests and diseases by using chemicals. It is the most common and quickest method among plant protection methods. In chemical control, various protective measures must be taken for humans, animals and the environment. Chemical pesticides are also called "pesticides". Pesticides are drugs made using chemical compounds or fillers that kill disease agents, pests and weeds that damage plants. Direct use of a pesticide against plants may cause phytotoxicity. In order to use them more safely and securely, they must be mixed with some auxiliary substances and fillers. This physical mixture is called formulation. To measure the amount of medicine to be used, the unit volume is determined by the amount of active substance in water. This is called "dose". Chemical control should be the last method used in combat methods. If pesticide control is to be carried out against any pest, it should be done with the appropriate pesticide targeting that pest, in the appropriate dose and at the most appropriate time (Anonymous, 2023a).

Classification of Chemical Control Pesticides According to Formulation Type

Liquid (liquid) formulations (EC)
Water-wettable powder formulations (WP)
Water-soluble powder formulations (SP)
Flowable formulations with the consistency of yoghurt (SC)
Powder formulations
Granular formulations
Pellet formulations
Gas formulations

8. INTEGRATED MANAGEMENT

It can be defined as "effectively protecting plants from the effects of diseases, pests and weeds, and minimizing the negative effects on the environment and human health by using all known methods in agricultural control together and balanced as much as possible (Delen et al., 2005). In other words it is the control of pests and diseases found in a particular agroecosystem and that the management against weeds should be done together, not separately, and that appropriate management methods and techniques should be used interchangeably. It is based on its implementation in a way that completes. In this struggle It is not desired to completely eliminate any living creature, whether beneficial or harmful. It is only aimed to keep the population densities of harmful factors below the economic damage level. Because of this, it is necessary to know about the economic damage thresholds of pests in integrated control programs. It is of great importance to protect and support the natural enemies present in nature (Karsavuran, 2005).

ABOUT FUNGUS

Like all other living things, fungi are named according to the "binomial" system and are named in Latin. In nomenclature, the first name is "genus" and the second name is "species". They constitute the largest group of plant pathogenic microorganisms. More than 100,000 fungal species are known today. More than 10,000 of these cause disease in plants. Fungi, formerly known as fungi, are microscopically small heterotrophic organisms that have thread-like and branched development and often have spore-bearing organs.

We can list the general characteristics of fungi as follows:

They are all eukaryotic.

They contain a nucleus containing chromosomes, bound to the cell membrane, and cytoplasmic organelles bound to the cell membrane.

Most of them have a filamentous structure.

They consist of microscopic individual filaments called hyphae, showing apical development, and structures called mycelium, which are formed by the branching development of these filaments.

Some are single-celled

Hyphae or cells are surrounded by a hard wall. This wall consists mainly of chitin and glucan, but some species contain cellulose in their walls.

Many reproduce both sexually and asexually.

Both sexual and asexual reproduction lead to spore formation. The nuclei of these spores are typically haploid, and the hyphal structures are often multinucleated. However, Oomycota and some yeasts contain diploid nuclei.

All of them are without chlorophyll. They do not contain chlorophyll pigment and cannot photosynthesize.

They are all chemoheterotrophic (chemo-organotrophic). They use the organic carbon resources available in their environment and obtain the energy necessary for their development and synthesis of organic matter from chemical reactions by breaking down this organic matter.

They contain characteristic storage compounds.

They can live freely or form close relationships with other organisms (Anonymous, 2023b).

Fungi that are pathogenic on plants are classified according to their lifestyle in and on their hosts.

1-endoparasite

2- ectoparasites.

Ectoparasites; fungal hyphae generally spread on the upper surface of the leaf and obtain their nutrients from the leaf epidermis with the help of haustorium, which are special feeding organs. Endoparasitic fungi develop inside the tissue. Development inside the tissue occurs either between cells or within cells. If growing within cells, the fungus obtains nutrients by absorption through the cell wall. If it grows between cells, the fungus extends its haustorium from its hyphae into the cell and provides its nutrients. Plant pathogenic fungi have developed different lifestyles and ways of interacting with host plants. Some pathogens kill the host cell with enzymes, reactive oxygen species and toxins after entering the plant (Horbach et al., 2011; Pawlowsk and Hartman, 2016; Gebrie, 2016). These are known as necrotrophic pathogens. Necrotrophic pathogens have a wide host spectrum and cause rapid tissue damage. Biotrophic pathogens are obligate parasites and do not produce toxins. They complete their life cycle only in the living host (Horbach et al. 2011; Pawlowsk and Hartman, 2016; Gebrie, 2016). In biotrophic pathogens, special infection structures are created to overcome the plant cell wall and enter the host cell. In ectoparasitic fungi, germination tubes are formed on the plant cuticle. The appressorial germination tube turns into structures called appressorium (Gebrie, 2016). Appressorium is a simple or protuberant swelling at the tip of the grass tube or hyphae that adheres to the food medium or host surface. It is a somatic structure that plays a role in clinging. In parasitic fungi, appressorium forms an "infection foot", which is a very thin hyphae formed under the host tissue after it adheres tightly to it and plays a role in penetrating the host tissue. If penetration is successful, the hook tips turn into houstoria. Housestorium are specialized structures formed within the living plant cell to obtain nutrients (Pawlowsk and Hartman, 2016). As the hyphal branch passes through the host cell wall, its diameter becomes very narrow, and after passing, it expands and becomes a simple or branched hoistorium. Hoistorium is the name given to the somatic structure that allows the intake of nutrients without killing the host cell. Necrotrophic fungi kill the host plant tissue by producing phytotoxins, cell wall-degrading enzymes. In necrotrophy, infection begins when the conidia germinate. To penetrate directly, it forms effection hyphae or to penetrate the epidermis, appressorium develops, forming penetration legs (Zelinger et al., 2016; Horbach et al., 2011; Pawlowsk et al., 2016; Gebre 2016). It secretes enzymes that degrade the host cuticle, such as appressorium oxidase, cutinase and lipase. The penetration spike kills cells by branching and producing cell wall-degrading enzymes. Thus, necrotic lesions are formed. Necrotrophs often produce phytotoxins to facilitate colonization. Although fungi are microscopic creatures, according to recent studies, fungi share a common ancestor with animals and show many similarities with animals in terms of evolution (Badouin et al., 2017). The fungi kingdom split off from its animal-common ancestor approximately 800-900 million years ago. Today, the fungi kingdom is considered one of the oldest and largest groups of living organisms (Moore et al., 2011). The taxonomy of fungi is changing with the acquisition of new genotypic data. For example, the Oomycetes class, of which more than 60% of its species are pathogens in plants, is no longer in the fungi kingdom (Thines and Kamoun, 2010; Albertin and Marullo, 2012). Although pathogens in the Oomycetes class are now included in the Chromista kingdom, they resemble fungi in the way they infect plants and in their characteristics such as flagellated development. Today, plant pathogenic fungi include a very large and heterogeneous group of organisms that are pathogenic on both agricultural and wild plants. These pathogens are of economic importance because they cause product losses in both agricultural fields and warehouses (Lo Presti et al., 2015). Plant pathogens have developed many mechanisms to bypass healthy host plant defense mechanisms. Genes that encode proteins that are possessed by the pathogenic organism and play a role in its pathogenicity are defined as avirulent genes (Avr genes). Identification and characterization of molecules involved in the interaction between host plant and pathogen have formed the basis of research over the last decade. The most important of the plant's defense mechanisms against diseases are R (resistant) genes. PR proteins (Pathogen Related Proteins), which are the products of R genes, are structural proteins that provide resistance against pathogens. PR proteins recognize and are stimulated by signal molecules that are the product of avirulent genes secreted by the pathogenic organism when it enters the plant. This mechanism is called the gene against gene hypothesis. Effector molecules synthesized by the Avr gene bind to the product of the R gene and activate it. The product of the activated R gene also provides the transmission of signals that will initiate the defense response. As a result of this interaction, rapid cell death is observed in neighboring cells around the infected cell. In this way, the spread of the pathogen in the host plant is prevented. This is called the Hypersensitive Response (HR) (Sexton and Howlett, 2006; Van der Does et al., 2007; Gonzalez Fernandez, 2010; Zelinger et al., 2010). The faster the host cell that interacts with the pathogen dies, the more resistant the plant is to infection (Sexton and Howlett, 2006; Van der Does et al., 2007; Zelinger et al., 2010). Understanding R gene systems molecularly will control diseases in crops. R-Avr proteins that regulate host-pathogen relationships in order to turn this relationship to his advantage. It is studied that Avr genes create mutations. Therefore, they have the same genotypic structure disease agent in areas where monoculture agriculture is practiced. The pathogen becomes virulent by mutating its Avr genes. As a result, all plants have diseases so epidemics occur. Considering the R-Avr gene relationships in agricultural production, different R varieties carrying genes or belonging to different plant populations different plant species should be planted in a polyculture manner. For permanent genetic resistance, the R genes of plants should be tested with the most virulent strains of the pathogen and R-Avr analyzes determining host-pathogen relationships should be performed by taking direct and indirect definitions into consideration. Genetic mapping, sequence analysis for the host's R genes and the structural features of the proteins they encode must be clarified (Burdon and Silk, 1997).

REFERENCES

Anonim, 2023a. https://www.tarimorman.gov.tr/GKGM/Belgeler/Tuketici_Bilgi_Kosesi/E-Bultenler/12.pdf (Access: December, 2023).

Anonim, 2023b. https://acikders.ankara.edu.tr/mod/resource/view.php?id=4760 (Access: December, 2023).

Albertin, W. ve Marullo, P. 2012. Polyploidy in fungi: evolution after whole-genome duplication. Proc. Royal Soc., 279, 2497-2509.

Badouin, H., Gladieux, P., Gouzy, J., Siguenza, S., Aguileta, G., Snirc, A., Le Prieur, S., Jeziorski, C., Branca, A. ve Giraud, T. 2017. Widespread selective sweeps throughout the genome of model plant pathogenic fungi and identification of effector candidates. Mol. Ecol., 26, 2041-2062.

Burdon, J. J. ve Silk, J. 1997. Sources and patterns of diversity in plant-pathogenic fungi. Phytopathol., 87 (7) 664-669.

Camacho, J. P. M., Sharbel, T. F. ve Beukeboom, L. W. 2000. B-chromosome evolution. Trans. Royal Soc. B, 355, 163178.

Çalış, Ö., 2011. Bitki Dayanıklılık Genleri ve Proteinleri. Gaziosmanpaşa Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü. Türk Bilimsel Derlemeler Dergisi 4 (2):1-9, 2011. ISSN: 1308-0040, E-ISSN: 2146-0132.

Delen, N., E. Durmuşoğlu, A. Güncan, N. Güngör, C. Turgut, A. Burçak, 2005. Türkiye'de pestisit kullanımı, kalıntı ve organizmalarda duyarlılık azalısı sorunları. Türkiye Ziraat Mühendisligi VI. Teknik Kongresi, 3-7 Ocak 2005, Cilt: 2. 629-648.

Gebrie S.A., 2016.Biotrophic Fungi Infection and Plant Defense Mechanisim, J Plant Pathol Microbiol, 7(9), 1-6.

Gonzalez-Fernandez R., Prats E., Jorrın-Novo J.V.J., Proteomcs of Plant Pathogenic Fungi, Journal of Biomedicine and Biotechnology, (Article ID 932527): 1-36.

Horbach R., Navarro-Quesada A.R., Wolfgang Knogge W., Deising H.B., 2010. When and how to kill a plant cell:Infection strategies of plant pathogenic fungi, Journal of Plant Physology, 168: 51–62 (2011).

Kadıoğlu, İ., 2012. Türkiye Tarımında Bitki Koruma ve Bazı Güncel Yaklaşımların Değerlendirilmesi. Gaziosmanpaşa Üniversitesi, Ziraat Fakültesi Bitki Koruma Bölümü-TOKAT. Temmuz-Aralık 2012, Sayı: 359

Karsavuran, Y., 2005. Entegre Mücadele Ve Temel İlkeleri, Örtü Altı Sebze Yetiştiriciliğinde Entegre Savaşım Kitabı, 25s-35s.

Ludwg-Müller J., 2015. Bactera and fung controllng plant growth by manpulatng auxn: Balance between development and defense, journal of plant physology,4-12.

Lo Presti, L., Lanver, D., Schweizer, G., Tanaka, S., Liang, L., Tollot, M., Zuccaro, A., Reissmann, S. and Kahmann, R., 2015. Fungal effectors ve plant susceptibility. Annu. Rev. Plant Biol., 66, 513-545.

Moore, D., Robson, G. D. ve Trinci, A. P. J., 2011. 21st century guidebook to fungi, Cambridge University Press, UK.

Pawlowski M.L., Hartman G.L., 2016. Infection Mechanisms and Colonization Patterns of Fungi Assocated with Soybean, Fungal Pathogencity, Sultan, S. (Ed.), InTech, DOI: 10.5772/62305. Available from:https://www.ntechopen.com/books/fungal-pathogenicty/infecton-mechanisms-andcolonzaton-patterns-of-fungi-assocated-with-soybean.

Ryder L.S., Talbot N. J., 2015. Regulaton of appressorum development n pathogenc Fung, Current Opnon n Plant Bology, 26:8–13.

Scharf D.H., Henekamp T., Brakhage A.A., 2014. Human and Plant Fungal Pathogens: The Role of Secondary Metaboltes, PLOS Pathogens, 10 (1): 1-3..

Sexton A.C., Howlett B.J., 2006. Parallels n Fungal Pathogenesis on Plant and Anmal Hosts, Eukaryotıc Cell, 5(12): 1941–1949.

Thines, M. ve Kamoun, S. 2010. Oomycete-plant coevolution: recent advances and future prospects. Curr. Opi. Plant Biol., 13, 427-433.

Van der Does H.C., Martjjn Rep M., 2007. Virulence Genes and the Evolution of Host Specificity in n PlantPathogenic Fungi, Mol Plant Microbe Interact , 20(10):1175-82.

Zeilinger S., Gupta V.K., Dahms T.E.S., Silva R.N., Singh H.B., Upadhyay R.S., Gomes E.V., Tsui C.K., Nayak S. C., 2016. Emergng insights from fungal interactons with plants, FEMS Microbology Reviews, 40(2):182-207.

CHAPTER 11

BIOCHEMICAL METHODS AND NANOTECHNOLOGICAL APPLICATIONS IN PLANT-MICROBE INTERACTIONS

Erdi Can AYTAR [1]
Alper DURMAZ[2]
Emine İncilay TORUNOĞLU[3]
Betül Arslan AYDIN[4]

DOI: https://dx.doi.org/10.5281/zenodo.10450486

---

[1] Department of Horticulture, Faculty of Agriculture, Usak University, Uşak, Türkiye erdi.aytar@usak.edu.tr Orcid ID: 0000-0001-6045-0183

[2] Botanical Garden Application and Research Center, Artvin Coruh University, Artvin, Türkiye alperdurmaz@artvin.edu.tr Orcid ID: 0000-0001-6927-3283

[3] Department of Medical Biochemistry, Faculty of Medical Meram, Necmettin Erbakan University, Konya, Türkiye torunogluemineincilay@gmail.com Orcid ID: 0000-0003-4641-0067

[4] Departman of Biology, Faculty of Science, Gazi University, Ankara,Türkiye. barslan@gazi.edu.tr Orcid ID: 0000-0002-9092-1350

INTRODUCTION

In recent years, the convergence of biochemical methodologies and nanotechnological advancements has presented innovative opportunities for investigating the intricate dynamics of biological systems, particularly focusing on plant-microbe interactions. The intricate interplay between plants and microbes plays a pivotal role in shaping ecosystems, agricultural productivity, and environmental sustainability. As researchers delve into the molecular intricacies of these interactions, the integration of biochemical approaches and cutting-edge nanotechnologies has emerged as a promising frontier.

Biochemical methods serve as valuable tools in unraveling the signaling pathways, metabolic responses, and molecular dialogues that define plant-microbe relationships. These methodologies guide researchers in identifying and quantifying essential biomolecules, shedding light on biochemical events that govern communication and coexistence between plants and microbes. Concurrently, the understanding of biochemical intricacies is complemented by nanotechnological applications, offering unparalleled opportunities to manipulate and engineer these interactions at the nanometer scale. Nanotechnology introduces a new paradigm for studying and modulating plant-microbe interactions by designing and controlling materials at the nanoscale. Nanoparticles, nanomaterials, and nanodevices provide unique advantages in targeting bioactive compounds, achieving controlled release, and enhancing the bioavailability of active components. The integration of nanoscale tools enables researchers to precisely intervene in cellular and molecular processes, providing insights into how plants respond to microbial stimuli.

This review explores the synergistic interaction of biochemical methodologies and nanotechnological applications in the context of plant-microbe interactions. By delving into the current state of knowledge, we highlight key findings arising from the integration of these multidisciplinary approaches. Furthermore, we discuss the potential implications of these findings for agriculture, environmental management, and the sustainable production of biological entities.

1.Plant Growth-Promoting Microorganisms (PGPMs)

Microorganisms that colonize plant roots in the rhizosphere and promote plant growth are called PGPMs (Vejan et al., 2016). PGPMs are known to be microbial partners of plants in the rhizosphere. They can play an important role in sustainable agriculture by improving soil quality, agricultural productivity, food and nutritional values, and agroecosystems (Abhilash et al., 2016). PGPMs encompass a diverse group of bacteria and fungi, that play a crucial role in enhancing plant growth and health. These microorganisms contribute to plant development through various mechanisms, including nutrient acquisition, hormonal regulation, and protection against pathogens.

1.1. Plant Growth-Promoting Bacteria (PGPB)

PGPB encompass a diverse range of microorganisms, including free-living bacteria, those forming specific symbiotic relationships with plants, and endophytic bacteria that may inhabit internal plant tissues. Additionally, cyanobacteria are considered part of this group (Vejan et al., 2016). Rhizospheric and plant tissue microbial communities employ distinct mechanisms to enhance plant growth. PGPB can directly stimulate plant growth by aiding in resource acquisition or by synthesizing plant hormones like auxins, cytokinins, and gibberellins, as well as growth regulators (Jalal et al., 2022). Moreover, they can indirectly promote growth by mitigating the inhibitory effects of various pathogens on plant development and growth (Glick, 2012). PGPB can be categorized into nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and plant growth-promoting rhizobacteria (PGPR), each serving unique roles in fostering plant health and vitality.

Nitrogen-fixing bacteria (NFB)

Nitrogen, which is essential for the survival of micro-organisms and plants, is not found in soil, but it is in the atmosphere. NFB convert atmospheric nitrogen into ammonia, making it available for plant uptake. They facilitate nitrogen fixation in root nodules (*Rhizobium* in legumes) or in the soil (*Azotobacter*, *Azospirillum*) (Olanrewaju et al., 2017; Jalal et al., 2022). NFB include symbiotic NF and non-symbiotic NFB (Lawal, 2021). The classification of nitrogen-fixing bacteria and their examples are shown in Table 1.

Table 1. Nitrogen-fixing bacteria

Nitrogen-fixing Type	Genus name
Symbiotic Nitrogen Fixing Bacteria	*Frankia* sp.
	Rhizobium sp.
	Sinorhizobium sp.
	Azorhizobium sp.
	Allorhizobium sp.
	Mesorhizobium sp.
	Bradyrhizobium sp.
	Azoarcus sp.
	Burkholderia sp.
	Herbaspirillum sp.
Non-Symbiotic Nitrogen Fixing Bacteria	*Azospirillum* sp.
	Paenibacillus sp.
	Herbaspirillum sp.
	Klebsiella sp.
	Pseudomonas sp.
	Enterobacter sp.
	Azotobacter sp.
	Clostridium sp.

Phosphate-Solubilizing Bacteria (PSB)

Phosphorus plays a vital role in metabolic activities and various functions and is an essential nutrient for plant growth and development. Phosphorus deficiency in plants can cause stunted growth, delayed crop maturity, and reduced seed formation (Billah et al., 2019). The soil often contains a substantial amount of insoluble phosphorus, rendering it inaccessible to plants and thereby limiting their growth. PSB release phosphorus bound in the soil, making it accessible to plants. They enhance plant growth by improving phosphorus uptake. Inorganic phosphorus is usually solubilized by the action of low-molar organic acids like gluconic and citric acids, both synthesized by various soil bacteria. The synthesis of various phosphatases, which catalyze the hydrolysis of phosphate esters, is responsible for the mineralization of organic phosphorus (Glick, 2012). Other phosphorus solubilization processes carried out by bacteria are exchange reactions, acidification, and chelation (Ramakrishna et al., 2019). Bacteria capable of phosphorus solubilisation are generally composed of bacteria belonging to the genera *Pseudomonas*, *Bacillus*, and *Rhizobium* (Siddique et al., 2021).

Plant growth-promoting rhizobacteria (PGPR)

Over the past decade, the rhizosphere has gained importance as an ecological niche in the biosphere, leading to increased research on the mechanics of PGPR in this area. PGPR are bacteria that live in the rhizosphere and improve the health of the plant, ultimately helping to increase the growth of the plant. Due to their suitability in specific environments, such as high or low temperatures and specific pH ranges, there is a wide range of PGPR that differ in their activities under varying environmental and soil conditions (Olanrewaju et al., 2017). There are several direct and indirect mechanisms by which PGPR can affect plant growth. Direct mechanisms of action of PGPRs include enhancing plant nutrition by supplying phytonutrients such as fixed nitrogen or solubilized phosphorus, potassium, zinc, iron, and other essential minerals from the soil as well as regulating the levels of phytohormones (Basu et al., 2021). Indirect mechanisms involve the plant's defensive metabolic pathways (Goswami et al., 2016).

Nitrogen fixation and phosphorus solubilization, which are the direct mechanisms of PGPRs, have been described above. Soil bacteria play a crucial role in providing iron to plants, especially when they are exposed to environmental stress, such as heavy metal pollution. In this case, siderophores relieve the stress on plants caused by high levels of heavy metals in the soil. Several studies have confirmed the direct benefits of bacterial siderophores on plant growth (Sultana et al., 2021; Sun et al., 2022; Zhang et al., 2023).

In regulating plant growth, development and responses to the environment, plant hormones play a crucial role. Plants commonly regulate their endogenous phytohormone levels to reduce the adverse effects of environmental stress factors when faced with growth-limiting environmental conditions. Although this strategy can be effective, it is important to note that rhizosphere microorganisms can produce or modulate phytohormones. As a result, many PGPR can influence phytohormone levels, thereby affecting the plant's hormonal balance and response to stress. Phytohormones mediate various processes in both symbiotic and non-symbiotic roots, including plant cell enlargement, division, and extension (Orozco-Mosqueda et al., 2023). Phytohormones synthesised by PGPR include auxins, cytokinins, and gibberellins (Mekureyaw et al., 2022; Nett et al., 2022; Iqbal et al., 2023).

2.The Role of Plant Growth-Promoting Rhizobacteria (PGPR) in Plant Development and Soil Health

The burgeoning global population constitutes a formidable challenge to agricultural food production, underscoring a critical imperative in addressing the sustenance needs of the rapidly expanding demographic. The escalating populace is directly impacted by multifarious factors, including constrained agricultural land, environmental degradation, and the exigencies imposed by biotic and abiotic stressors, collectively exerting discernible ramifications on global food production. The imperative to augment agricultural productivity becomes paramount in meeting the alimentary demands of the burgeoning populace. Diverse strategies are requisite, encompassing heightened recourse to chemical inputs such as fertilizers, pesticides, and herbicides. Concurrently, the deployment of microorganisms conducive to stress-tolerant plant growth, particularly in saline and arid terrains, emerges as an integral facet for amplifying agricultural productivity. It is worth noting, however, that extant solutions to this predicament are frequently ephemeral in efficacy and lack long-term sustainability. PGPR, as exemplified by microorganisms proficient in synthesizing bioactive compounds such as gibberellins and indole acetic acid, assumes centrality in the promotion of stress-resilient plant growth. Under adverse conditions typified by drought, heavy metal exposure, and salinity stress, PGPR manifests heightened enzymatic activity. The PGPR paradigm holds promise in not only amplifying plant growth but also in perpetuating crop yield sustainability within an ecologically conscientious milieu. The intricate interplay between plants and microorganisms imparts salience to the augmentation of plant health, growth dynamics, nutrient bioavailability, and assimilative capacities, thereby fortifying the plant's resilience against an array of pathogenic entities. These reciprocal interactions are epitomized by the synthesis of diverse phytohormones, including cytokinins, auxins, gibberellins, and ACC-deaminase. The latter, ACC-deaminase, assumes significance in mitigating ethylene levels within nascent plant roots. Additional activities encompass symbiotic nitrogen fixation, mineral solubilization, and ancillary mechanisms that bolster plants in navigating stressors. The symbiotic interplay between microbial agents within the plant rhizosphere, culminating in positive modulations of plant growth dynamics, substantiates an augmentation of stress tolerance in the face of adverse environmental exigencies. (Kumar et al., 2019)

PGPR delineate a subset of bacteria that adeptly establish themselves within the root systems of plants, exhibiting the capacity to augment both plant growth and yield. This bacterial cohort demonstrates a notable ability to colonize the entirety of ecological niches within root ecosystems across all stages of plant development, even in the presence of a competitive microfloral milieu. In contemporary discourse, the nomenclature "PGPR" encompasses all bacteria inhabiting the rhizosphere that, through one or multiple mechanisms, contribute to the enhancement of plant growth. Varied species of rhizobacteria, affiliated with genera including *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus,* and *Serratia*, have been designated as PGPR.

The modus operandi of these bacteria typically involves augmenting nutrient assimilation, modulating levels of plant hormones, and mitigating the inhibitory impacts of diverse pathogens on plant growth, thereby functioning as biocontrol agents. Furthermore, PGPR showcase an environmental detoxification aptitude, effectively cleansing the surroundings by eliminating pollutants such as heavy metals and pesticides. The multifaceted effects of these bacteria are contingent upon ecological nuances, soil attributes, plant classifications, age, developmental stages, and soil composition. In broad strokes, PGPR bacteria play a pivotal role in positively regulating plant growth and enhancing agricultural sustainability by fine-tuning plant-soil dynamics. (Prasad et al., 2019).

In a 2023 research investigation, the impact of PGPR inoculations was scrutinized in the context of Moso bamboo cultivation. The applications of PGPR manifested a notable enhancement in Moso bamboo biomass, ranging between 34.2% and 108.9%. Concurrently, discernible improvements were observed in soil nutrient content and enzyme activities. The microbial diversity witnessed an augmentation, coupled with a reduction in the abundance of

Bacteroidetes. Significantly, mixed bacterial inoculants demonstrated efficacy in preserving the stability of the soil microbial community. The study further revealed pronounced effects on the activation of genes associated with phosphorus solubility, specifically phoA, phoD, and phoN. These findings collectively underscore the affirmative influence of PGPR inoculations on Moso bamboo growth, concomitant with a stabilizing effect on the microbial community. (Li et al., 2023).

In a subsequent investigation, the pre-exposure of wheat to a specific PGPR strain was observed to elicit a cascade of beneficial effects. Notably, this pre-exposure regime instigated a discernible surge in vegetative growth, encompassing pronounced increases in both shoot and root lengths, total leaf area, and the biomass of both above-ground and below-ground components. Beyond the realms of morphological enhancements, wheat pre-exposed to the PGPR strain exhibited an augmented resistance against B. sorokinana, indicating a fortification of the plant's defense mechanisms. Upon closer examination of physiological parameters, a robust elevation was documented in photosynthetic pigments, indicative of an intensified photosynthetic activity. Furthermore, there were conspicuous elevations in both enzymatic and non-enzymatic antioxidant activities, reflective of an enhanced capacity to counteract oxidative stress. The overall reactive oxygen species (ROS) scavenging activities were notably heightened in comparison to non-pre-exposed wheat plants. Importantly, both PGPR strains demonstrated a comparable efficacy in conferring protection to wheat plants, signifying their consistent performance in this regard. Intriguingly, a detailed biochemical analysis illuminated that the pre-exposure to PGPR did not immediately incite a direct defense response. This nuanced finding implies a strategic utilization of resources, where the plant refrains from initiating defense mechanisms in the absence of stress, thus averting unnecessary resource allocation. As the wheat plants reached maturity, the dividends of pre-exposure became even more apparent. Pre-exposed wheat exhibited a superior yield performance when contrasted with non-pre-exposed counterparts. However, the scope of the study expanded beyond the immediate generation, as a compelling revelation emerged: the positive effects of pre-exposure transcended the initial generation and were robustly transmitted to the subsequent generation. Progeny originating from pre-exposed wheat showcased not only heightened disease resistance but also superior productivity when subjected to disease pressure compared to their non-pre-exposed counterparts. This observation underlines the enduring and transgenerational impact of PGPR pre-exposure in bolstering plant resilience and productivity under pathogenic stress. (Devi et al., 2023)

The established understanding in the scientific literature underscores that PGPR not only play a pivotal role in promoting plant growth and enhancing resistance but also exhibit stimulatory or inductive effects on specific plant traits. Among these discernible traits is the phytoremediation capacity inherent in plants. Contemporary environmental challenges are exemplified by the escalating threat posed by polycyclic aromatic hydrocarbons (PAHs) to both soil ecosystems and human health, substantiating their deleterious accumulation in terrestrial environments. This predicament accentuates the environmental predicaments stemming from the unrestrained release and accrual of PAHs. The cumulative presence of PAHs in soil carries the dual risk of impinging upon plant growth, thereby imperiling agricultural productivity, and undermining the biological diversity integral to soil ecosystems. Moreover, the repercussions of these pollutants on human health are exacerbated due to their potential transmission through the food chain. In this scholarly milieu, the utilization of PGPR, known for their prowess in enhancing plant growth, emerges as a strategic avenue for effective intervention in the phytoremediation of soils contaminated with PAHs. Pursuant to this objective, the Serratia sp. DLN5 strain, distinguished by its capability to degrade phenanthrene (PHE), was judiciously chosen. A meticulous evaluation of this strain's impact on plant physiology and phytoremediation efficacy ensued. The findings delineate that DLN5 inoculation substantively amplifies the growth of canola plants cultivated in PAH-contaminated soil, concurrently enhancing phytoremediation efficiency. Furthermore, the regulatory effects of this activity on plant growth and soil microbial communities were scrutinized, thereby laying the groundwork for an avant-garde PGPR-supported phytoremediation strategy poised for practical application in soils beset by PAH contamination. (Guan et al., 2023).

In conclusion, the integration of PGPR into agricultural practices stands as a promising solution for fostering an ideal and sustainable farming system. This strategic use not only contributes to the improvement and preservation of human health but also yields economic and spiritual advantages for both producers and consumers. Furthermore, PGPR plays a crucial role in addressing the increasing global demand for food production while simultaneously ensuring environmental sustainability. The primary impediments to global agricultural productivity lie in the challenges posed by biotic and abiotic stress factors within environmental conditions. Plant-associated microorganisms, particularly stress-tolerant PGPRs, assume a pivotal role in imparting resistance to these stressors. Stress-tolerant PGPRs exhibit versatile functionality, encompassing mechanisms that enhance crop yield, control environmental pollution, and establish an eco-friendly environment within the framework of sustainable development. These mechanisms involve nitrogen fixation initiation, phosphate solubility assurance, growth hormone release, and the deployment of elements such as siderophores and osmotic responses. Consequently, PGPRs not only contribute to the reduction of excessive chemical and pesticide usage but also minimize human health risks associated with the residual effects of these substances. Additionally, the application of PGPR enhances soil nutrient balance, thereby sustaining the productivity of agricultural lands in a balanced and sustainable manner. Given these multifaceted benefits, the incorporation of PGPR into agricultural strategies emerges as a promising avenue for fostering a resilient and sustainable global food production system.

3. Importance of Antioxidant Mechanism During Plant-Microbe Interaction

Plants, due to their stationary structures, are exposed to biotic and abiotic stress factors (Dar et al., 2022). In response to these stresses, plants generate fundamental signaling molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). These molecules play a critical role in regulating metabolism, growth, development, and defense mechanisms against various stress factors in plants. Various biotic and abiotic stress factors, such as intense light, excessive temperature, salt, drought, waterlogging, and plant pathogens, trigger ROS and RNS production in plants (Imran et al., 2021). In plant cells, various cellular oxidation processes take place for growth, development, and coping with stress. These oxidation reactions result in by-products called ROS. The normal functionality of plant cells is defined by the ability to maintain the delicate balance between ROS production and clearance.

The interaction of plants with microbes disrupts this delicate balance by triggering excessive ROS production, leading to oxidative stress. This oxidative stress is associated with programmed cell death, stomatal closure, gravitropism, and the development of other responses, and ROS has been identified as a secondary messenger in intracellular signal transduction pathways (Choudhary & Upadhyay, 2023). It has been noted that microbes are widespread, diverse, and adaptable organisms to complex environments, and they have extensive metabolic processes to alleviate abiotic stress conditions. Plant-microbe interactions are considered a significant component of living ecosystems that assist plants in initiating defense mechanisms under adverse environmental conditions in nature (Meena et al., 2017)

Plants establish symbiotic relationships with non-pathogenic or beneficial microorganisms in their root systems. However, pathogenic microorganisms can have detrimental effects, increasing the formation of ROS and posing a risk of toxicity. In response to such challenges, plants activate their natural defense systems. Symbiotic interactions have been reported to support plant defense mechanisms by enhancing the production of phytohormones and defense-related molecules. Antioxidants, including enzymes such as catalase, and superoxide dismutase, as well as non-enzymatic compounds like tocopherols, carotenoids, and glutathione, play a crucial role in supporting plant defense mechanisms against ROS and contribute to the survival of plants under stressful conditions. The interaction between pathogens and plants influences both acquired and induced defense mechanisms. Through a comprehensive antioxidant network, plants can mount a more effective defense against pathogens (Singh et al., 2021).

Amid challenging environmental conditions, the enzyme superoxide dismutase (SOD) plays a pivotal role in mitigating oxidative damage by swiftly converting superoxide anions ($O_2−$) into oxygen molecules (O_2) and hydrogen peroxide (H_2O_2). SOD functions as a crucial enzymatic system tasked with eliminating free radicals ($O_2−$) generated by plants under stress. It collaborates closely with enzymes such as catalases (CAT) and peroxidases (POX) to suppress the production of reactive oxygen species (ROS) from $O_2−$ and H_2O_2 through the Haber-Weiss reaction. In addition to H2O2 breakdown and maintaining redox homeostasis, POX eliminates H2O2 by catalyzing the oxidation of phenolic substrates, wherein H_2O_2 acts as an electron acceptor.

Ascorbate peroxidase (APX) contributes to the conversion of H_2O_2 within the cytoplasm and other cellular structures—a process facilitated by POX and CAT in the ascorbate-glutathione cycle. Malondialdehyde (MDA) is accountable for the oxidative breakdown of polyunsaturated fatty acids in plant cell membranes (Ummara et al., 2022).

The predominant antioxidant metabolite in plant cells is ascorbic acid, also known as vitamin C (Celi et al., 2023). The function of APX involves direct interaction with various ROS, neutralizing the deleterious effects of O_2−, -OH, and O_2, while actively participating as an electron donor in enzymatic activities that reduce H_2O_2 levels (Hasanuzzaman et al., 2020). In a study by Castro et al., it was revealed that at elevated levels of ascorbic acid, particularly in the presence of intense H_2O_2 levels, ascorbic acid could act as a prooxidant, triggering the Fenton reaction in rice leaves exposed to intense UV radiation and thereby increasing oxidative stress (Castro et al., 2018).The increase in antioxidant activity can be expected to be a significant indicator of stress management in plant-microbe interactions. CAT, POX, and phenylalanine ammonia-lyase (PAL) play a role in cell wall elongation through the polymerization of glycoproteins rich in lignification, suberification, and hydroxyproline, among other functions. Antioxidants also play a role in wound healing, in addition to their involvement in plant-microbe interaction resistance reactions (Choudhary & Upadhyay, 2023). While the generation of reactive oxygen species (ROS) is commonly linked with pathogen invasion, ROS production has also been noted in alternative biotic interactions, such as those involving bacteria or mycorrhiza. This suggests that the synthesis of ROS is a prevalent characteristic in various biotic relationships. The observation that ROS synthesis is low and antioxidant system levels are high after exposure to beneficial microorganisms indicates a distinctive characteristic of plant responses (Sahu et al., 2022). To protect themselves from pathogen attacks and infections, plants utilize mechanisms such as hypersensitive response (HR)-mediated cell death, apoptosis lignin biosynthesis, pattern-triggered immunity (PTI), and gene-mediated effector-triggered immunity (ETI). The representation of ROS in PTI, ETI, and symbiotic connections is illustrated in Figure 1.

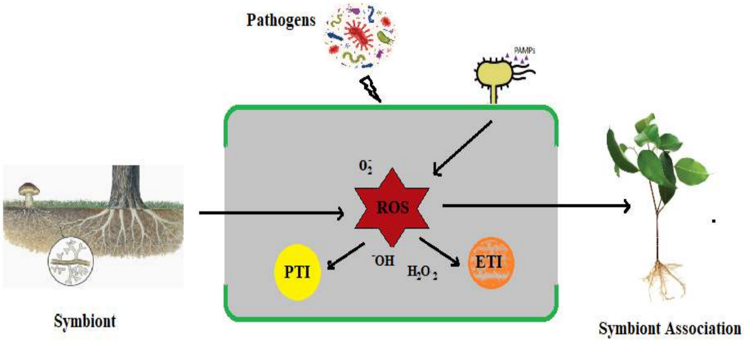


Figure 1: A graphical depiction illustrating ROS within PTI, ETI, and symbiotic connections.

In conclusion, the production and regulation of signaling molecules such as ROS and RNS play a crucial role in the effective adaptation mechanisms of plants against various stress factors. These signals assist plants in coordinating growth, development, defense, and survival strategies.

4. Nanotechnological Advancements in Plant-Microbe Interaction

Nanotechnology is an evolving field of the 21st century, and nanoparticles, when compared to their larger counterparts (1-100 nm), exhibit distinct structural, optical, electrical, magnetic, and mechanical properties in the nanoscale. In the last two decades, the interest in transition metal oxide nanoparticles has increased due to emerging applications in various fields such as catalysis, biosensors, cosmetics, pharmaceuticals, food and agriculture, electronics, dentistry, energy, and the environment (Javed et al., 2022; Katwal et al., 2015). Copper oxide (CuO) nanoparticles (NPs) demonstrate various impressive features in biomedical fields and serve as potent bactericidal, catalytic, anti-carcinogenic, and coating agents. They possess an approximate 2 eV bandgap energy and have gained significant importance due to their chemical inertness and thermal stability (Grigore et al., 2016; Naz et al., 2023).

4.1 Nanoparticle Properties and Synthesis Methods

The qualities of nanoparticles are determined by a combination of their physical and chemical characteristics. Physical properties include optical, mechanical, suspension, sedimentation, thermal, magnetic, and electrical features. Optical properties, for instance, are closely tied to the sizes of noble metal nanoparticles, showcasing a distinctive UV-visible absorption band in their spectra. This phenomenon, known as localized surface plasmon resonance (LSPR), occurs when the frequency of incident photons resonates with the collective excitation of conduction electrons. The peak wavelength of the LSPR spectrum varies based on the size, shape, and interparticle distance of nanoparticles.

Mechanical properties encompass attributes like flexibility, elasticity, and tensile strength. These mechanical features play a crucial role in diverse research fields such as tribology, surface engineering, nanofabrication, and nanomanufacturing (Dhaka et al., 2023; Strambeanu et al., 2014). These properties enable researchers to delve into various aspects of nanoparticle behavior and performance, contributing to advancements in multiple scientific and technological domains.

The magnetic and electrical properties of nanoparticles stem from the distorted electronic distribution. Magnetic and electrical characteristics are determined through terms such as conductivity, semi-conductivity, and resistance. These features make nanoparticles applicable in contemporary electronic applications. Additionally, essential attributes include thermal conductivity, suspension, hydrophilicity, hydrophobicity, and diffusion. The role of nanoparticles in chemistry and biological engineering is governed by their chemical properties. These properties, influenced by factors like stability, sensitivity, toxicity, oxidation, reduction, and chemical compositions with antifungal and antibacterial attributes, varies according to the nanoparticle sizes (Anwar et al., 2021).

Biologically synthesized nanoparticles offer several advantages, including non-toxicity, high production efficiency, easy scalability, and well-defined morphology. Consequently, an innovative method for nanoparticle production is essential. The green synthesis technique has been developed as an effective approach for nanoparticle synthesis. Nanoparticles synthesized through green methods are characterized by safety, environmental friendliness, and ease of use (Vijayaram et al., 2023). The synthesis of green nanoparticles is categorized into two classes based on nanoparticle formation: "top-down" and "bottom-up" approaches. In the "top-down" approach, nanoparticles have a larger size, and methods such as mechanical techniques or acid addition are employed to reduce their size. Generally, the top-down approach necessitates intricate analyses (thermal separation method, mechanical method/ball milling method, lithographic methods, laser ablation, spray). The "bottom-up" approach significantly differs from the top-down process and commences with the assembly of molecules at the atomic level. Bottom-up methods are executed through various techniques (chemical vapor deposition (CVD) method, sol-gel method, spinning, pyrolysis) (Ijaz et al., 2020).

4.2 Agricultural Applications of Microbe-Synthesized Nanoparticles (NPs)

While many NPs are being employed as antimicrobial agents for treating various diseases in living organisms, the utilization of NPs in the treatment of diverse plant diseases is still in its early stages. NPs have the potential to facilitate targeted transfers to specific regions within plants. The monitoring of the endogenous movement of NPs proves to be highly advantageous for the directed treatment of specific plant regions (Naidu et al.,2023). Earlier studies have elucidated the use of NPs for transporting biomolecules into plant cells, utilizing their magnetic properties to guide localization. For instance, research conducted on *Cucurbita pepo* showcased the *in vitro* application of NPs, employing confocal, optical, and electron microscopes to detect localization. NPs combined with macromolecules like nucleotides, drugs, and enzymes can effectively facilitate gene formation in complex delivery systems, supporting transfer and expression within host cells (Ali et al., 2021).

In the agricultural sector, there is significant interest in the production of functional NPs with properties of affordability, compatibility, and biological biodegradability. These NPs can serve as nano-pesticides, herbicides, nano-fertilizers, nano-sensors, herbicides, and nano-additives .Over the past few years, nanotechnology has become more widespread in the agricultural industry, aiming to reduce the use of environmentally harmful chemicals, employ nano-sensors for accurate and highly sensitive detection, enhance the effectiveness of primary plant protection methods, and develop strategies to reduce abiotic and harmful effects in biotic stresses (Spanos et al., 2021).

In a study conducted by Yan et al. (2020), interactions between Fe_3O_4 NPs and maize plants were investigated using a combination of phenotypic and metabolic approaches. *Zea mays* were grown for four weeks in an environment where Fe_3O_4 NPs were processed in treated soil at concentrations of 0, 50, and 500 mg/kg. There was no significant effect of Fe_3O_4 NPs on plant biomass or photosynthesis. However, a significant increase in root length and a decrease in malondialdehyde (MDA) levels were observed, indicating positive effects on root development and membrane integrity. Inductive coupled plasma optical emission spectrometry (ICP-OES) analysis showed that Fe_3O_4 NPs led to significant iron accumulation in roots rather than leaves. Furthermore, 500 mg/kg Fe_3O_4 NPs increased dehydrogenase enzyme activity by 84.9%. Metabolomic analysis revealed that exposure of maize roots to Fe_3O_4 NPs rendered antioxidant and defense-related metabolic pathways ineffective. These results suggest that Fe_3O_4 NPs may play a protective role against microbes and induce specific metabolic changes in plant roots. The findings of this study provide new molecular mechanisms to assess environmental impacts and understand the response of maize plants to Fe_3O_4 NPs (Yan et al., 2023).

The diverse characteristics and applications of nanoparticles, as discovered through various studies, mentioned above, can propel significant advancements in future research within the fields of nanotechnology and biotechnology. The highlighted features of these nanoparticles have the potential to enhance effective and sustainable solutions in various industrial and biomedical applications.

The biosynthesis and green synthesis methods of nanoparticles can enable environmentally friendly and cost-effective production processes, thus facilitating the broader-scale utilization of nanomaterials. This has the potential to offer effective solutions across a wide spectrum, ranging from the agricultural sector to biomedical applications.

CONCLUSIONS

The convergence of these themes emphasizes the intricate interaction network that determines plant-microbe relationships, including the role of nanoparticles. Contributions range from the macro-level effects of plant growth-promoting microorganisms in agricultural applications to the micro-level complexities of antioxidant responses and the influence of nanoparticles in these dynamics.

Navigating at the forefront of agricultural and environmental sciences, the integration of these insights, including nanoparticle effects, holds promise for more sustainable, resilient, and efficient ecosystems. The collective knowledge derived from these focal points serves as a foundation for future research triggering innovations in agricultural practices and environmental management.

REFERENCES

Abhilash, P.C., Dubey, R.K., Tripathi, V., Gupta, V.K., Singh, H.B. (2016). Plant Growth-Promoting Microorganisms for Environmental Sustainability. Trends in Biotechnology, 34, 847–850. https://doi.org/10.1016/j.tibtech.2016.05.005

Ali, A., Shah, T., Ullah, R., Zhou, P., Guo, M., Ovais, M., & Rui, Y. (2021). Review on recent progress in magnetic nanoparticles: Synthesis, characterization, and diverse applications. Frontiers in Chemistry, 9, 629054.

Anwar, A., Yousuf, M. A., Zulfiqar, S., Agboola, P. O., Shakir, I., Al-Khalli, N. F., & Warsi, M. F. (2021). The impact of highly paramagnetic Gd3+ cations on structural, spectral, magnetic and dielectric properties of spinel nickel ferrite nanoparticles. Journal of Saudi Chemical Society, 25(9), 101306.

Basu, A., Prasad, P., Das, S.N., Kalam, S., Sayyed, R.Z., Reddy, M.S., El Enshasy, H. (2021). Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. Sustainability 13, 1140. https://doi.org/10.3390/su13031140

Billah, M., Khan, M., Bano, A., Hassan, T.U., Munir, A., Gurmani, A.R. (2019). Phosphorus and phosphate solubilizing bacteria: Keys for sustainable agriculture. Geomicrobiology Journal, 36, 904–916. https://doi.org/10.1080/01490451.2019.1654043

Castro, J.L.S., Lima-Melo, Y., Carvalho, F.E.L., Feitosa, A.G.S., Lima Neto, M. C., Caverzan, A., Margis-Pinheiro, M., & Silveira, J.A.G. (2018). Ascorbic acid toxicity is related to oxidative stress and enhanced by high light and knockdown of chloroplast ascorbate peroxidases in rice plants. Theoretical and Experimental Plant Physiology, 30(1), 41–55. https://doi.org/10.1007/S40626-018-0100-Y/FIGURES/6

Celi, G.E.A., Gratão, P.L., Lanza, M.G.D.B., & Reis, A.R. dos. (2023). Physiological and biochemical roles of ascorbic acid on mitigation of abiotic stresses in plants. Plant Physiology and Biochemistry, 202, 107970. https://doi.org/10.1016/J.PLAPHY.2023.107970

Choudhary, P., & Upadhyay, P. (2023). Relevance of the antioxidative mechanism during plant-microbe interaction. Plant-Microbe Interaction - Recent Advances in Molecular and Biochemical Approaches: Volume 1:

Overview of Biochemical and Physiological Alteration During Plant-Microbe Interaction, 123–140. https://doi.org/10.1016/B978-0-323-91875-6.00014-1

Dar, F.A., Mushtaq, N.U., Saleem, S., Rehman, R.U., Dar, T.U.H., & Hakeem, K. R. (2022). Role of Epigenetics in Modulating Phenotypic Plasticity against Abiotic Stresses in Plants. International Journal of Genomics, 2022. https://doi.org/10.1155/2022/1092894

Devi, B., Tiwari, M., Yadav, N., & Singh, P. (2023). Intergenerational immune priming: Harnessing plant growth promoting rhizobacteria (PGPR) for augmented wheat protection against spot blotch. Physiological and Molecular Plant Pathology, 128, 102164. https://doi.org/10.1016/J.PMPP.2023.102164

Dhaka, A., Mali, S.C., Sharma, S., & Trivedi, R. (2023). A review on biological synthesis of Silver nanoparticles and their potential applications. Results in Chemistry, 101108.

Glick, B.R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. Scientifica, 1–15. https://doi.org/10.6064/2012/963401

Goswami, D., Thakker, J.N., Dhandhukia, P.C. (2016). Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food & Agriculture, 2, 1127500. https://doi.org/10.1080/23311932.2015.1127500

Grigore, M.E., Biscu, E.R., Holban, A.M., Gestal, M.C., & Grumezescu, A. M. (2016). Methods of synthesis, properties and biomedical applications of CuO nanoparticles. Pharmaceuticals, 9(4), 75.

Guan, C., Fu, W., Zhang, X., Li, Z., Zhu, Y., Chen, F., Ji, J., Wang, G., & Gao, X. (2023). Enhanced phytoremediation efficiency of PHE-contaminated soil by rape (Brassica napus L.) assisted with PHE-degradable PGPR through modulating rhizobacterial communities. Industrial Crops and Products, 202, 117057. https://doi.org/10.1016/J.INDCROP.2023.117057

Hasanuzzaman, M., Bhuyan, M.H.M.B., Zulfiqar, F., Raza, A., Mohsin, S.M., Al Mahmud, J., Fujita, M., & Fotopoulos, V. (2020). Reactive Oxygen Species and Antioxidant Defense in Plants under Abiotic Stress: Revisiting the Crucial Role of a Universal Defense Regulator. Antioxidants, 9(8), 1–52. https://doi.org/10.3390/ANTIOX9080681

Ijaz, I., Gilani, E., Nazir, A., & Bukhari, A. (2020). Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. Green Chemistry Letters and Reviews, 13(3), 223-245.

Imran, M., Shazad, R., Bilal, S., Imran, Q.M., Khan, M., Kang, S.M., Khan, A. L., Yun, B. W., & Lee, I.J. (2021). Exogenous Melatonin mediates the regulation of endogenous nitric oxide in Glycine max L. to reduce effects of drought stress. Environmental and Experimental Botany, 188, 104511. https://doi.org/10.1016/J.ENVEXPBOT.2021.104511

Iqbal, M., Naveed, M., Sanaullah, M., Brtnicky, M., Hussain, M.I., Kucerik, J., & Mustafa, A. (2023). Plant microbe mediated enhancement in growth and yield of canola (Brassica napus L.) plant through auxin production and increased nutrient acquisition. Journal of Soils and Sediments, 23(3), 1233-1249.

Jalal, A., Filho, M.C.M.T., da Silva, E.C., da Silva Oliveira, C E., Freitas, L.A., & do Nascimento, V. (2022). Plant Growth-Promoting Bacteria and Nitrogen Fixing Bacteria: Sustainability of Non-legume Crops. In Nitrogen Fixing Bacteria: Sustainable Growth of Non-legumes (pp. 233-275). Singapore: Springer Nature Singapore.

Javed, A., Shah, A.H., Hussain, A., Khan, S.A., Khan, S.A., Hamayun, M., & Jan, S. A. (2019). Identification and characterization of Penicillium chrysogenum T8 as potent plant growth promoting endophytic fungi. FEB-Fresenius Environmental Bulletin, 4896.

Javed, R., Ain, N.U., Gul, A., Arslan Ahmad, M., Guo, W., Ao, Q., & Tian, S. (2022). Diverse biotechnological applications of multifunctional titanium dioxide nanoparticles: An up-to-date review. IET nanobiotechnology, 16(5), 171-189.

Katwal, R., Kaur, H., Sharma, G., Naushad, M., & Pathania, D. (2015). Electrochemical synthesized copper oxide nanoparticles for enhanced photocatalytic and antimicrobial activity. Journal of Industrial and Engineering Chemistry, 31, 173-184.

Kumar, A., Patel, J.S., Meena, V.S., & Srivastava, R. (2019). Recent advances of PGPR based approaches for stress tolerance in plants for sustainable agriculture. Biocatalysis and Agricultural Biotechnology, 20, 101271. https://doi.org/10.1016/J.BCAB.2019.101271

Lawal, I. (2021). Nitrogen Fixing Bacteria and Their Application for Heavy Metal Removal: A Mini Review. Journal of Biochemistry, Microbiology and Biotechnology, 9, 43–47. https://doi.org/10.54987/jobimb.v9i2.617

Li, N., Wen, J., Wu, R., Hu, D., Zhang, L., Zhang, W., & Zhang, M. (2023). Dual effects of plant growth-promoting rhizobacteria (PGPR) on the Moso bamboo-soil system: Plant growth promotion and microbial community stability. Industrial Crops and Products, 203, 117151. https://doi.org/10.1016/J.INDCROP.2023.117151

Meena, K.K., Sorty, A.M., Bitla, U.M., Choudhary, K., Gupta, P., Pareek, A., Singh, D.P., Prabha, R., Sahu, P.K., Gupta, V.K., Singh, H.B., Krishanani, K. K., & Minhas, P. S. (2017). Abiotic Stress Responses and Microbe-Mediated Mitigation in Plants: The Omics Strategies. Frontiers in Plant Science, 8. https://doi.org/10.3389/FPLS.2017.00172

Mekureyaw, M.F., Pandey, C., Hennessy, R.C., Nicolaisen, M.H., Liu, F., Nybroe, O., & Roitsch, T. (2022). The cytokinin-producing plant beneficial bacterium Pseudomonas fluorescens G20-18 primes tomato (Solanum lycopersicum) for enhanced drought stress responses. Journal of Plant Physiology, 270, 153629.

Naidu, S., Singh, I.K., & Singh, A. (2023). Microbial Synthesis of Magnetic Nanoparticles for Plant Science and Agriculture. Plant Nano Biology, 100036.

Naz, S., Gul, A., Zia, M., & Javed, R. (2023). Synthesis, biomedical applications, and toxicity of CuO nanoparticles. Applied Microbiology and Biotechnology, 107(4), 1039-1061.

Nett, R.S., Bender, K.S., & Peters, R.J. (2022). Production of the plant hormone gibberellin by rhizobia increases host legume nodule size. The ISME Journal, 16(7), 1809-1817. https://doi.org/10.1038/s41396-022-01236-5

Olanrewaju, O.S., Glick, B.R., Babalola, O.O. (2017). Mechanisms of action of plant growth promoting bacteria. World Journal of Microbiology and Biotechnology, 33. https://doi.org/10.1007/s11274-017-2364-9

Orozco-Mosqueda, MdC., Santoyo, G., Glick, B.R. (2023). Recent Advances in the Bacterial Phytohormone Modulation of Plant Growth. Plants, 12(3), 606. https://doi.org/10.3390/plants12030606

Prasad, M., Srinivasan, R., Chaudhary, M., Choudhary, M., & Jat, L.K. (2019). Plant Growth Promoting Rhizobacteria (PGPR) for Sustainable

Agriculture: Perspectives and Challenges. PGPR Amelioration in Sustainable Agriculture, 129–157. https://doi.org/10.1016/B978-0-12-815879-1.00007-0

Ramakrishna, W., Yadav, R., & Li, K. (2019). Plant growth promoting bacteria in agriculture: Two sides of a coin. Applied Soil Ecology, 138, 10-18.

Sahu, P. K., Jayalakshmi, K., Tilgam, J., Gupta, A., Nagaraju, Y., Kumar, A., Hamid, S., Singh, H.V., Minkina, T., Rajput, V.D., & Rajawat, M.V.S. (2022). ROS generated from biotic stress: Effects on plants and alleviation by endophytic microbes. Frontiers in Plant Science, 13, 1042936. https://doi.org/10.3389/FPLS.2022.1042936/BIBTEX

Siddique, R., Gul, A., Ozturk, M., & Altay, V. (2021). Phosphate Solubilizing Bacteria for Soil Sustainability. Handbook of Assisted and Amendment: Enhanced Sustainable Remediation Technology, 423-435.

Singh, A.K., Kumar, S., & Sinha, T. (2021). Antioxidants in Plant–Microbe Interaction. Antioxidants in Plant-Microbe Interaction, 3–20. https://doi.org/10.1007/978-981-16-1350-0_1/COVER

Spanos, A., Athanasiou, K., Ioannou, A., Fotopoulos, V., & Krasia-Christoforou, T. (2021). Functionalized magnetic nanomaterials in agricultural applications. Nanomaterials, 11(11), 3106.

Strambeanu, N., Demetrovici, L., Dragos, D., & Lungu, M. (2014). Nanoparticles: Definition, classification and general physical properties. In Nanoparticles' Promises and Risks: Characterization, Manipulation, and Potential Hazards to Humanity and the Environment (pp. 3-8). Cham: Springer International Publishing.

Sultana, S., Alam, S., & Karim, M.M. (2021). Screening of siderophore-producing salt-tolerant rhizobacteria suitable for supporting plant growth in saline soils with iron limitation. Journal of Agriculture and Food Research, 4, 100150.

Sun, Y., Wu, J., Shang, X., Xue, L., Ji, G., Chang, S., & Emaneghemi, B. (2022). Screening of siderophore-producing bacteria and their effects on promoting the growth of plants. Current Microbiology, 79(5), 150.

Ummara, U., Noreen, S., Afzal, M., Zafar, Z.U., Akhter, M.S., Iqbal, S., Hefft, D.I., Kazi, M., & Ahmad, P. (2022). Induced systemic tolerance mediated by plant-microbe interaction in maize (Zea mays L.) plants under hydrocarbon contamination. Chemosphere, 290, 133327. https://doi.org/10.1016/J.CHEMOSPHERE.2021.133327

Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., Nasrulhaq Boyce, A. (2016). Role of Plant Growth Promoting Rhizobacteria in Agricultural Sustainability—A Review. Molecules, 21, 573. https://doi.org/10.3390/molecules21050573

Vijayaram, S., Razafindralambo, H., Sun, Y.Z., Vasantharaj, S., Ghafarifarsani, H., Hoseinifar, S.H., & Raeeszadeh, M. (2023). Applications of Green Synthesized Metal Nanoparticles—A Review. Biological Trace Element Research, 1-27.

Yan, L., Li, P., Zhao, X., Ji, R., & Zhao, L. (2020). Physiological and metabolic responses of maize (Zea mays) plants to Fe3O4 nanoparticles. Science of the Total Environment, 718, 137400.

Zhang, S., Deng, Z., Borham, A., Ma, Y., Wang, Y., Hu, J., & Bohu, T. (2023). Significance of Soil Siderophore-Producing Bacteria in Evaluation and Elevation of Crop Yield. Horticulturae, 9(3), 370.

Zhao, X., Ni, Y., Zhao, H., Liu, X., He, B., Shi, B., & Liu, H. (2021). Plant growth-promoting ability and control efficacy of Penicillium aurantiogriseum 44M-3 against sesame Fusarium wilt disease. Biocontrol Science and Technology, 31(12), 1314-1329.

İKSAD
Publishing House

ISBN: 978-625-367-581-3