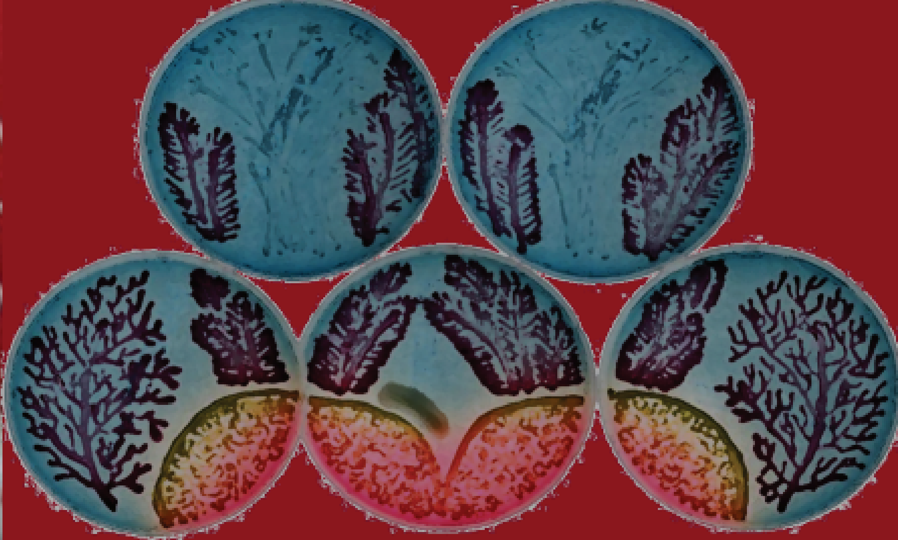
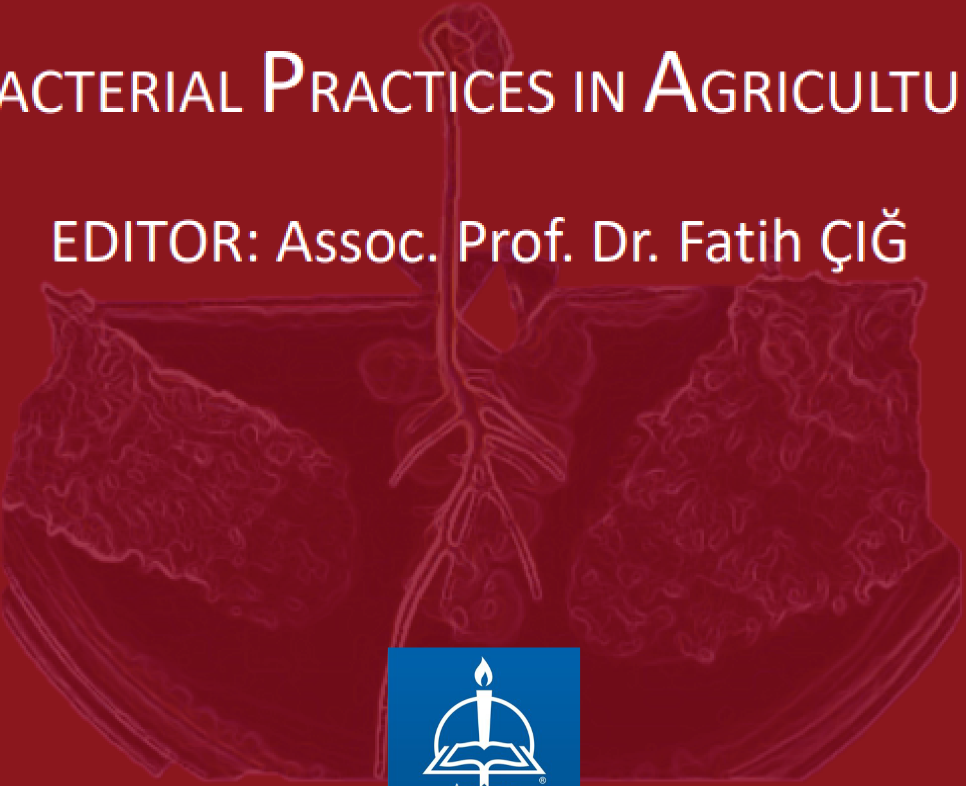


BACTERIAL PRACTICES IN AGRICULTURE

EDITOR: Assoc. Prof. Dr. Fatih ÇIĞ



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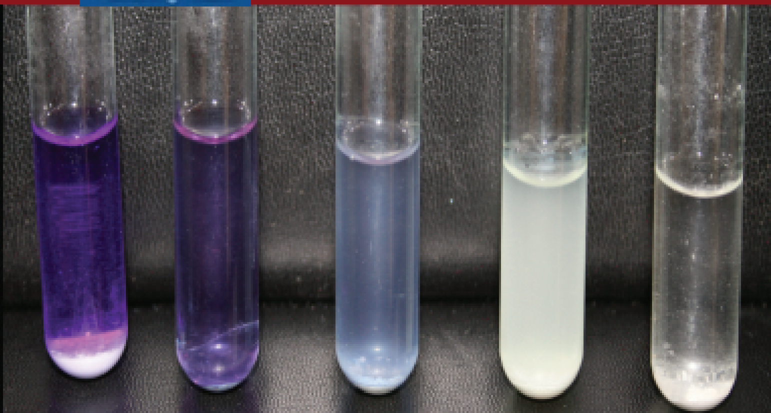

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CONTENTS

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PREFACE

CHAPTER 1

ISOLATION AND APPLICATIONS OF BENEFICIAL MICRORGANISMS ASSOCIATED WITH TEA PLANTS (*Camellia sinensis* L.)

CHAPTER 2

A SUSTAINABLE AND EFFECTIVE STRATEGY IN SALINITY STRESS MANAGEMENT: PGPB-PRIMING

CHAPTER 3

INTERACTION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) WITH MEDICINAL AND ORNAMENTAL PLANTS

PREFACE

For addressing the challenges of ever-increasing world's population, the maximization of farming crops production are of key challenges to think through for sustaining global food security. The aim of this book was to gain insight into potential differences and novel application of bacterial practices in agriculture and it is provides an overview of new trends in bacteria exploitation in crop production and sustainable agriculture. The objective of this book is to in depth study of the bacterial applications in modern agriculture and understanding their uses in future global food security without losing of agroecosystems sustainability. The increase in the world needs for food poses a challenge to our ability to enhance soil fertility. The using of bacteria, adopted in different areas of the world as an alternative technique, could contribute to decrease the erosion of soils and improving in the soil properties and crop productivity. The contribution of bacterial applications in sustainable agriculture will be expanded for sustainable agriculture. The advance technology and strategies in farming system to explore these natural and friendly resource for of plant and soil health and new application of bacteria are also discussed.

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CHAPTER 1

ISOLATION AND APPLICATIONS OF BENEFICIAL MICRORGANISMS ASSOCIATED WITH TEA PLANTS (*Camellia sinensis* L.)

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INTRODUCTION

There is a growing interest in the use of plant-associated microorganisms, which play a central role in soil and plant health, as well as plant nutrition through carbon and nutrient cycling, and are becoming increasingly important for agricultural sustainability. Beneficial, free-living, rhizosphere and root-colonizing bacteria that stimulate plant growth, increase yield, and reduce stress are generally considered plant growth-promoting rhizobacteria (PGPR). PGPR operate and effect on plants through direct and indirect mechanisms or a combination of both, and there by minimize the environmental impact of chemical intensive farming practices (Chauhan et al., 2015). PGPR enhance plant growth by a wide variety of mechanisms like biological N_2- fixation, solubilization and mineralization of organic/inorganic P, K, Zn and Fe, production of siderophore, ammonia, salycylic acid, and phytohormones like indole acetic acid (IAA), cytokinins, gibberellins, ethylene, certain volatiles etc., producing plant growth regulators and volatile organic compounds, recycling of essential elements and uptake of essential nutrients from the soil, organic matter mineralization, rhizosphere engineering, soil structure formation, containing of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, lowering of plant ethylene level, quorum sensing signal interference and reducing biofilm formation, degrading organic pollutants, improving plant-microbe symbioses, and indirectly acting as a biocontrol agents by mechanisms such as producing siderophores, β-1,3-glucanase, chitinases, antibiotics, fluorescent pigment and cyanide, and inducing systemic resistance (Çakmakçı et al., 2006, 2017a; Hayat et al., 2010; Bhattacharyya & Jha, 2012; Glick, 2012; Chauhan et al., 2015; Gupta et al., 2015; Çakmakçı, 2019a). The use of free-living beneficial bacteria as an important agricultural input to increase crop production requires the selection of competent rhizobacteria with plant growth-promoting properties. The use of PGPR may be more important, especially in plants where leaves are used, and since only leaves are used in tea processing, such bacteria are becoming more and more important in sustainable tea production (Çakmakçı et al., 2014). Indeed, PGPR applications to tea plants found to affect survival rate, plant growth, biometric parameters, nutrient use efficiency, enzymes and systemic resistance (Thomas et al., 2010).

Tea is a popular herb grown commercially in more than 50 countries in well-distributed rainy, hot, and humid climates, well-drained, deep, fairly loamy, acidic and lime-free soils, and its shoot used as a beverage. Since it increases leaf yield, tea needs more nitrogen compared to other plants and its requirement has increased year after year. In addition, fertilizers may not be effective in this regard due to runoff and leaching under the high rainfall tea ecosystem. In long-term continuous cropping tea garden systems, tea bushes grow very close together and root secretions can accumulate from all sides in the rhizosphere, causing a significant inhibitory effect, even some nutrient content accumulates in the soil, soil quality in the root zone decreases and tea growth is inhibited (Çakmakçı, 2019a).

Soil quality, fertility and health of tea plantation systems, especially biological parameters, depend on agricultural management practices, and long-term overuse of inorganic fertilizers adversely affects them. The composition, abundance, and structure of microbial communities in the soil ecosystem are affected by environmental factors such as pH, temperature, humidity, and soil nutrient availability, as well as farm management practices such as tillage, crop rotation, fertilization and pesticide application (Chen et al., 2021). The inoculum efficiency of microbial products, which play an important role in sustainable agriculture, varies depending on a number of factors such as soil mineral content, type of crop and competition with native strains. As an alternative, the use of bacterial inoculants can contribute to increasing agricultural productivity and solving environmental problems by reducing the use of chemical, environmental pollution and production costs if the selected and developed bacterial inoculants are efficient. This review discusses various microorganisms especially bacteria, their ecology, isolation, characterization, strategies, principles, future aspect, nutrient uptake and use efficiency, microbial diversity and rhizosphere microbe interactions in tea cropping systems, processes involved in the efficiency, and applications for tea and future directions.

1. BACTERIAL DIVERSITY AND MICROBES INTERACTIONS IN TEA CROPPING SYSTEMS

Plant-microbe associations in the rhizosphere play an important role in ecosystem processes, plant ecology and performance, ecosystem functioning, sustainability of the ecosystem, and regulation of nutrient cycling. In the old tea gardens according to the mixed tea orchard systems with legumes, natural naturally grown tea plants in forests and forest soils (Çakmakçı, 2019b). In young tea plants, the bacterial population in the rhizosphere soil was higher than in the non-rhizosphere, while the number of bacteria in the tea soil was reduced in long-term continuous crop tea garden systems. Long-term overuse of fertilizers in tea plantation created a highly selective habitat, decreased pH, and negatively affected microbial diversity and richness. Intensive use of chemical fertilizers and long-term monoculture cause functional diversity changes in the composition of the beneficial bacterial community, reduce the functions of the rhizosphere microbiota, change the microbial community structure and biochemical and biological attributes of the soil, and reduce microbial diversity (Ai et al., 2015; Mortimer et al., 2015; Li et al., 2016; Çakmakçı, 2019c). Chemical fertilizer application can increase crop yield, but fertilizers become poorer in soil organic matter day-by-day, decrease biological activity with increasing acidity, change soil properties and bacterial community structure, and increase heavy metal uptake of tea. Despite all this, investigation of the effect of PGPR on tea plants and soils as well as the response of fertilizer management on bacterial community structure in tea plantations is still weak and very inadequate (Çakmakçı et al., 2017b; Wang et al., 2019).

Bacterial composition in the plant rhizosphere changes according to soil properties, climate, plant species, and varietal and ecotype differences between species. Since plants can control and change the ecology, they grow in with the microbial community in their rhizosphere, the density, quality and characteristics of the bacterial population in the rhizosphere soils of cultivated and wild plants that have been in the same region for many years are different. Although the rhizospheric bacterial population was higher in young tea plants than in the non-rhizosphere soil depending on age, the pH of the tea bushes soils gradually decreased and the microbial community structure in the rhizosphere soils changed with increasing age after the establishment of the garden (Wang et al., 2010; Çakmakçı, 2019c). Lowering of the soil pH with age was found to be an important factor resulting in the suppression of microbial communities in the tea rhizosphere (Pandey & Palni, 1997). Root exudates are the main factor affecting biological efficiency in and around the rhizosphere, and after tea roots reach a certain age, they begin to secrete antimicrobial metabolites to which bacteria are most sensitive. On the other hand, instability of soil nutrients, the toxicity of root exudates, reduction of ammonia-oxidizing bacteria and changes in the microbial community are increasing in tea gardens (Lin et al., 2012). Taking advantage of the beneficial role of microorganisms in improving nutrient availability in the rhizosphere is a sustainable beneficial strategy for agriculture. Plant roots release metabolites such as organic acids, amino acids, saccharides, phenolics, flavonoids and terpenoids into the rhizosphere, which leads to the multiplication of microorganisms around the root zone, which causes the multiplication of microorganisms around the root zone. Root exudates influence all microbiota around the nearby soil, and there is an important variation in bacterial diversity in the rhizosphere soils of different plant varieties.

Total N, organic C and available K content have the greatest influence on soil bacterial diversity, which is a sensitive indicator of soil quality and sustainable cultivation use (Wang et al., 2021).

It was reported that while the availability of N, P, K, Ca^{2+} and Mg^{2+} decreased in acidic soils, Fe, Ni, Cu, Zn, Mn and Al were found more and their solubility increased and could cause toxicity (Nath, 2014). In the tea-Chinese chestnut intercropping system, enrichment of soil with organic matter, available P and K showed a positive correlation with bacterial taxa such as *Myxococcota*, *Latescibacterota, Bacteroidota, Chloroflexi, Deinococcota, Bdellovibrionota,* and *Patescibacteria* (Wu et al., 2021). Although the response of nutrients and microbial populations in long-term tea orchards with various agroforestry intercropping systems are not fully understood, the abundance of *Firmicutes, Actinobacteria, Proteobacteria*, and *Bacteroidetes*, which are dominant in all intercropping systems, showed a positive relationship with available N, soil organic matter and total P (Zhang et al., 2021). Soil organic matter, NH4+-N and available P were found to be the most important factors affecting the abundance and diversity of nitrogen-fixing bacteria in the tea soils. In another study, although not between soil bacterial populations and pH and organic carbon, a significant positive correlation was found between ammonifying bacteria with *Nitrosomonas* and *Nitrobacter*, and *Nitrosomonas* with *Nitrobacter* (Gafur & Sultana, 2013). The rhizosphere of the resident tea bushes has specific characteristics determined by the agricultural practices and tea plant itself, such as negative rhizosphere effect, lowering of soil pH, interaction between bacterial communities, and dominance of specific species (Çakmakçı, 2019b). The rhizosphere effect of different tea varieties is high in tea orchards and the soil microbial community associated with plant cultivars.

Wang et al. (2019) and Yang et al. (2021) found that *Proteobacteria* was the most common phylum, followed by *Actinobacteria*, *Chloroflexi* and *Acidobacteria* among bacterial phyla in the rhizosphere of cultivated tea bushes. Although bacterial diversity in the tea soils is closely related to environmental factors such as water content, pH, nutrient abundance, organic carbon, and total nitrogen, *Acidobacteria, Bacteroidetes, Actinobacteria, Chloroflexi, Gemmatimonadetes, Proteobacteria, Planctomycetes* and *Verrucomicrobia* are the most common phyla found in tea plant rhizosphere soil (Wang et al., 2021).

Mostly, *Proteobacteria* are characterized as free-living and root-associated N_2-fixing *Betaproteobacteria* and aerobic anoxygenic phototrophic bacteria that provide carbon and nitrogen for efficient and sustainable tea cultivation (Diagne et al., 2017; Zheng et al., 2011). The use of integrated fertilizers strongly changed the level and composition of bacterial phyla and genus compared to the use of chemical fertilizers, and it was shown that bacterial phyla *Actinobacteria, Bacteroidetes, Nitrospirae, Latescibacteria,* and *Saccharibacteria*, and genera were mainly *Bradyrhizobium, Flavobacterium, Haliangium, Nitrospira, Roseiflexus,* and *Ramlibacter* (Wang et al., 2019). The rhizosphere and rootzone of tea have been reported to be a good habitat for PGPR strains represented by *Bacillus*, *Proteus*, and *Pseudomonas*, inhibitory to phytopathogenic fungi, such as *Fusarium oxysorum, F. udum,* and *F. solani* (Kumar & Bezbaruah, 1996; Verma et al., 2010). The diversity of bacterial populations was examined in 1, 10 and 20-year tea orchard soils and proved that *Gammaproteobacteria*, *Alphaproteobacteria*, *Acidobacteria* and *Actinobacteria* were the most abundant phyla in tea soils and comprised more than 60% of bacterial sequences (Li et al., 2016). As a result, important reductions in soil pH, organic matter, and nutrient content, as well as the relative plenty of beneficial bacteria such as *Pseudomonas, Rhodanobacter, Bradyrhizobium, Mycobacterium*, and *Sphingomonas*, have been reported after 20 years of continuous monoculture.

The occurrence and activity of soil microorganisms are affected by a variety of environmental factors such as soil type, pH, nutrient abundance, moisture content, as well as plant-related factors. The associated heterotrophic rhizobacteria of the rhizoplane and rhizosphere depend on the carbohydrates exuded by the plant roots. Plants play an important role in selecting and enriching the type of bacteria by the constituents of their root exudates. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties and relies on soil biological processes and soil biodiversity.

2. BACTERIA ISOLATION TEA PLANTATION

Many promising culturable PGPR associated with tea has been isolated and used as biofertilizers; however, their effects on crop production fluctuate from crop to crop, site to site, and from season to season, depending on the effectiveness and survival of the applied microorganisms in the root rhizosphere soil. The culturable PGPR isolated so far from tea rhizosphere belong mainly to *Bacillus subtilis, B. cereus, B. megaterium, B. mycoides, B. licheniformis, B. sphaericus, B. atrophaeus, Paenibacillus polymyxa, Enterobacter aerogenes, Micrococcus luteus, Serratia marcescens, Stenotrophomonas maltophilia, Pseudomonas fluorescens, P. putida, Pseudomonas nitroreducens, Pseudomonas stutzeri, Burkholderia cepacia, B. pyrrocinia, Alcaligenes faecalis, Azotobacter chrococcum, Azospirillum brasilense, Azospirillum lipoferum* (Pandey & Palni, 1997; Shanmugam et al., 2008; Saravanakumar et al., 2007; Çakmakçı et al., 2010a; Bagyalakshmi et al., 2012a; Princy et al., 2014; Yang et al., 2014; Dutta & Thakur, 2017, Khan et al., 2017; Bhattacharyya & Sarmah, 2018; Purkayastha et al., 2018; Varmazyari & Çakmakçı, 2018; Bhattacharyya et al., 2020; Han et al., 2021), but other species suh as *Acinetobacter* sp. (Wafula et al., 2014), *Agrobacterium radiobacter*, *Pseudomonas aeruginosa*, *P. hunanensis,* and *Bacillus flexus* (Wang & Han, 2019), *Azospirillum* sp., *Microbacterium* sp. and *Rhodococcus* sp. (Tennakoon et al., 2019), *Bacillus* sp., *B. flexus, B. pseudomycoides, Lysinibacillus macroides, L. fusiformis,* and *L. sphaericus* (Khan et al., 2017), *Bacillus aryabhattai* (Bhattacharyya et al., 2017), *Bacillus safensis* and *Luteibacter* sp. (Sun et al., 2019), *Brevibacterium* sp. (Chopra et al., 2020), *Brevibacterium casei*, *B. celere*, *B. bullata*, *Microbacterium lacticum, Micrococcus yunnanensis* and *Kocuria marina* (Wei et al., 2018), *Herbaspirillum* sp. (Cheng et al., 2017), *Paenibacillus camelliae* (Oh et al., 2008), *Paenibacillus* sp.

(Khan et al., 2017; Liu et al., 2017), *Pseudomonas* sp. (Kumar & Bezbaruah, 1996; Verma et al., 2007), *P. aeruginosa* (Morang et al., 2012; George et al., 2015), *Pseudorivibacter rhizosphaerae* (Zhou et al., 2020a), *S. marcescens, B. megaterium, B. amyloliquefaciens* and *B. pumilus* (Chakraborty et al., 2010, 2012, 2013, 2015), *Streptomyces* (Shan et al., 2018; Dutta & Thakur, 2020) have also been identified. On the other hand, the culturable potassium solubilizing bacteria isolated so far from tea rhizosphere and growing soil belong mainly to *B. subtilis, B. pseudomycoides, P. nitroreducens, P. putida,* and *B. cepacia* (Bagyalakshmi et al., 2012b, 2017; Princy et al., 2014; Han et al., 2018; Pramanik et al., 2019).

Although limited, some *Azospirillum, Azotobacter, Achromobacter, Bacillus, Mycobacterium, Clostridium,* and *Beijerinkia* species have been reported to have nitrogen-fixing abilities in early research (Natsvaladze et al., 1992). Based on the phospholipid fatty acid (PLFA) profiles (Xue et al., 2008) and fatty acid methyl ester (FAME) and substrate utilization profiles (Çakmakçı et al., 2010a; Gulati et al., 2011; Varmazyari & Çakmakçı, 2018; Çakmakçı, 2019c) revealed the presence of both Gram-positive and Gram-negative bacteria within the rhizosphere soils of tea orchards although higher number was that of Gram-positive. In previous studies, cultivated dominant Gram-positive genera (*Bacillus, Paenibacillus, Arthrobacter, Brevibacillus, Microbacterium, Kurthia, Rhodococcus, Kocuria,* and *Micrococcus*) and Gram-negative (*Pseudomonas, Stenotrophomonas, Brevundimonas, Alcaligenes, Acinetobacter, Enterobacter, Rhizobium,* *Mesorhizobium, Burkholderia, Flavobacterium, Xanthomonas, Lysobacter, Azospira, Delftia, Serratia, Erwinia, Herbaspirillum, Ralstonia¸ Caulobacter*, *Chromobacterium*, *Azospirillum*, *Azotobacter*) were isolated from the acidic tea rhizosphere (Sood et al., 2008; Çakmakçı et al., 2010a; Gulati et al., 2011; Varmazyari & Çakmakçı, 2018; Yan et al., 2018; Bora et al., 2021). Many species of bacteria belonging to the genera *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Stenotrophomonas*, *Arthrobacter*, *Serratia*, *Brevibacillus,* and *Burkholderia* were found to be the most abundant N_2-fixing and P-solvent groups in the acidic tea soil of high rainfall, temperate regions (Çakmakçı et al., 2010a; Varmazyari & Çakmakçı, 2018). In addition, certain bacterial genera, such as *Bacillus, Burkholderia, Pseudomonas, Acinetobacter, Chryseobacterium, Enterobacter, Serratia* and *Micrococcus*, were often found in high populations in tea growing areas that were also shown to have the ability to fix atmospheric nitrogen and solubilize phosphorous (Wafula et al., 2014).

Gram-positive *Bacillus* and *Paenibacillus*, and Gram-negative *Pseudomonas* species were found in large numbers in the plant rhizosphere soils of tea orchards, as well as the predominance of Gram-positive bacteria (Xue et al., 2008; Çakmakçı et al., 2010a; Varmazyari & Çakmakçı, 2018). The genetic diversity of previous studies has revealed that *Bacillus* is the most predominant bacterial genera found in tea soils (Çakmakçı et al., 2010a; Dutta & Thakur, 2017; Goswami et al., 2017). Phylogenetic analysis results in previous research have reported the predominance of Gram-positive bacteria, including

Bacillus and *Paenibacillus*, under the *Firmicutes* phylum in tea orchards (Gulati et a., 2011; Han et al., 2014). Growth promoting *Azospirillum*, *Beijerinckia*, *Pseudomonas* and *Bacillus* species were isolated from tea plants, and the tea rhizosphere was found to be a good habitat for PGPR represented by *Bacillus* and *Pseudomonas* species (Phukan et al., 2012). Similarly, in some previous studies *Proteobacteria, Actinobacteria* and *Firmicutes* were the predominant phyla detected in tea orchard soils (Xue et al., 2006; Zhao et al., 2012; Yuan et al., 2017), whereas others found *Bacillus* followed by *Pseudomonas* as the dominant genera (Sood et al., 2008; Çakmakçı et al., 2010a; Varmazyari & Çakmakçı, 2018). Khan et al. (2017) found that *Bacillus* (63%) was the most dominant genus in the tea rhizosphere soil, followed by *Lysinibacillus* (19%), *Paenibacillus* (12%), and *Brevibacterium* (6%). *Bacillus* species play a vital role in N fixation and P, K, Zn and Fe cycles as one of the most dominant rhizospheric bacteria/rhizobacteria that increase plant growth through different mechanisms (Pandey & Palni, 1997; Çakmakçı et al., 2010a; Saxena et al., 2020).

In the previous study, 124 bacterial strains were isolated, which belonged to 31 genera and 54 species, from 51 natural tea waste samples from 17 locations, and characterize them for plant growth promoting attributes out of which 103 strains were efficient in N_2-fixing activity, 85 were efficient in P-solubilization and 76 were effective in N_2-fixation and P-solubilization (Çakmakçı & Varmazyari, 2018). Among the N_2-fixing and P-solubilizing cultivated bacteria in tea wastes, *B.*

licheniformis, B. cereus, B. pumilus, B. laevolacticus, B. subtilis, B. megaterium and *P. polymyxa* are the dominant gram-positive bacterial species, while *P. fluorescens, P. putida* and *S. maltophilia* are a Gram-negative bacterial species in tea wastes. These bacteria, which are isolated from tea waste in the natural environment, have the biotransformation of complex organic substrates into useful decomposed residues and can also accelerate the composting process, which could be used in potential industrial applications.

By using different selective media and methods, a total of 110 isolates in three phylum (*Firmicutes, Proteobacteria* and *Bacteroidetes*) from Zijuan and 164 isolates in two phylum (*Firmicutes* and *Proteobacteria*) from Yunkang were obtained by Yan et al. (2018) and their PGP activities were investigated by different methods. Most isolates of *Herbaspirillum* and *Brevundimonas* and a few *Methylobacterium* isolates showed the N fixing activities, and bacterial populations and PGP activities changed depending on different seasons and tea varieties. In another study, 46 actinobacteria belonging to 11 families and 13 genera were obtained from leaf, stem and root samples of tea, and their IAA and ACC ability were determined in 93.5% and 21.7%, respectively (Shan et al., 2018). In a study examining beneficial bacteria in forest, young, mature and high-altitude tea gardens, when *Proteobacteria* was the dominant phylum and *Bradyrhizobium* was the predominant genus in all soil's samples, a total of 27 phyla, 49 classes, 93 orders, 169 families and 337 genera showed nitrogen fixing ability (Chen et al., 2020). Of thirty bacterial strains representing nine different genera, which were isolated from rhizospheric soils of seven tea plantations, all of them were found to produce IAA, ammonia, and siderophore and solubilize phosphates, 12 of them were able to exhibit ACC deaminase activity, and 21 and 8 of them were able to produce protease and cellulase, respectively (Bhattacharyya et al., 2020). In the previous study, 271 bacterial strains were isolated from tea soil were characterized for PGP attributes out of which 106 strains were efficient in P solubilization, 66 isolates exhibited IAA production, 65 isolates showed siderophore and 164 were positive for ammonia production, and 50 strains were showed efficient for all the four traits (Dutta et al., 2015).

Over one thousand four hundred and thirty dominant, morphologically different rhizobacteria belonging to a total of 63 genera and 122 species were isolated and purified from 580 rhizospheric soil samples of tea crops grown in 62 locations in the Eastern Black Sea Region and were evaluated their potential use for P activity, high carbon sources utilization and improving plant growth of tea (Çakmakçı et al., 2010b, 2011; Çakmakçı, 2019c). Out of a total of 786 bacterial strains selected and assessed, 424 strains were found to have N_2-fixing, 335 to P-solubilizing, 98 to ACC deaminase activity, and 285 to N_2-fixing and P-solubilizing activity. Single, double, triple and quadruple formulations formed from 460 bacteria selected from the rhizobacteria pool were tested for PGP and enzymes activity enhancing the potential of tea clones such as Hayrat, Fener-3, Muradiye-10, Tuğlalı-10, Gündoğdu, Pazar-20, and Hamzabey under natural soil conditions by conducting in greenhouse, pot and field trials between 2006-2017 years, and it was concluded that a total of 98 strains belong to 45 species could be evaluated in plant production and used in biological fertilizer formulations (Çakmakçı et al., 2010a, 2010b, 2011). Inoculation with IAA and ACC deaminase producer, phosphate solubilizer and nitrogen fixing bacteria based formulations stimulated overall tea plant growth, including shoot and root development, plant height, trunk diameter, leaf area and yield, nutrient uptake, chlorophyll value, and activities of enzymes such as GR, GST, G6PD, 6PGD, PPO, POD, urease, DHSK, and ADH, but it was strongly dependent on strain specific properties and consortia, and parameters evaluated (Çakmakçı et al., 2012, 2013, 2014, 2015, 2017b, 2017c, 2018).

Borah et al. (2019) characterized the native 129 isolates from tea rhizospheric soil, and then tested in vitro for specific PGP traits, such as the production of IAA, siderophore, ammonia, extracellular enzyme and P solubilization. According to 6S rRNA sequencing and blast analysis, these isolates were found to belong to *Bacillus, Brevibacterium, Paenibacillus* and *Lysinibacillus* genera. Likewise, Shan et al. (2018) isolated 13 genera such as *Streptomyces, Actinomadura, Kytococcus, Kribbella, Leifsonia, Microbacterium, Micromonospora, Mycobacterium, Nocardia, Mobilicoccus, Nocardiopsis, Piscicoccus*, and *Pseudonocardia* from young leaf, stem, and lateral root in tea collected from seven tea plantations. These isolates, identified based on 16S RNA sequencing analysis, showed positive activity for IAA, ACC deaminase, and antibacterial, antifungal, and bioactive metabolites, while they exhibited inhibitory activity against one bacterial or fungal pathogen, metabolic potential to produce secondary metabolites, and PGP properties.

Endophytes can be defined as all organisms that live in the organs of plants such as flowers, leaves, stems, roots and can colonize internal plant tissues at any period of their life without causing significant damage to the host. As endophytic bacteria and fungi regulate not only host plant growth but also resistance to stresses, it appears to be a promising solution for sustainable agriculture as a natural reservoir against climate change. Tea plants contain plenty in endophytic fungi and bacteria, endophytic bacteria contain 24 families and 32 genera, and especially *Micrococcales, Bacillales, Rhizobiales* and *Burkholderiales* bacterial strains and *Pleosporales, Diaporthales, Glomerellales, Hypocreales* and *Xylariales* fungal strains were reported to be the dominant species (Xie et al., 2020). Endophytic strains can be isolated mainly from the roots, shoots, stems, leaves and flowers of tea plants. Recently, 129 endophytic bacteria were isolated from leaf and root samples of six *Camellia* species, BLAST analysis and 16S rRNA sequencing showed that they belonged to genera such as *Bacillus, Brevibacterium, Paenibacillus* and *Lysinibacillus* (Borah et al., 2019). Among these bacterial strains, 61 strains exhibited P-solubilizing activity, while IAA, ammonium, and siderophore producing isolates were 42, 66, and 33, respectively. Of the 23 isolates isolated from rhizospheric tea soils by Chopra et al. (2020), 10, 17 and 23 indicated positive activity for phosphate solubility, IAA and ammonia production, respectively, while 11, 17, 14 and 7 isolates were found to have antagonistic effects against *R. solani*, *Fomes lamaensis*, *Corticium rolfsii*, and all three fungal pathogens, respectively. Eighteen endophytic bacterial strains isolated from tea roots were tested for their PGP activities, and all were IAA producers, twelve were ammonia producers, 7 were siderophore producers, and 5 were found phosphate solubilizer (Nath et al., 2013). Wei et al. (2018) isolated 44 endophytic actinomycetes belonging to 12 genera such as *Brevibacterium, Brachybacterium, Kocuria, Mycobacterium, Micrococcus, Leucobacter, Streptomyces, Microbacterium, Pseudarthrobacter*, and *Saccharomonospora* from leaves of two tea cultivars. Wei et al. (2018) reported that the most common endophytic actinomycetes in tea leaves were *Brevibacterium*, whereas Shan et al. (2018) found that in the leaves, new stems and lateral roots of tea plants was dominated by *Streptomyces* genera of the endophytic actinomycetes. *Streptomyces* was previously reported as the most plentiful endophytic actinobacterial genus with varied PGP traits from leaves and roots of *Camellia* species (Borah & Thakur, 2020). Moreover, in a study conducted by Dutta and Thakur (2020) a *Streptomyces* sp. TT3 isolated from rhizosphere of established tea plants was able to exhibit antagonistic activity against fungal pathogens in tea through IAA, ammonia and siderophore production, phosphate solubilization, and actinorhodin and ethyl acetate production. Recently, *Herbaspirillum camelliae* isolated from ornamental tea plants was able to produce IAA, ammonia and siderophore as well as phosphorus solubilizing capacity (Liu et al., 2020).

Recently, the isolation of endophytic actinobacteria from tea plants by cultivation-based methods was reported, with the major genera obtained being *Bacillus, Herbaspirillum, Methylobacterium, Pantoea, Acinetobacter, Sphingomonas, Staphylococcus, Variovorax, Serratia, Ralstonia*, and *Fictibacillus* (Yan et al., 2018; Liu et al, 2020). Bora et al. (2021), in their study on the isolation of bacteria from the organically grown *Camellia* population, the class-level taxonomic hits distribution was showed that approximately 41, 20, 14, 11, 5, 1 and 2% bacteria belong to *Proteobacteria, Acidobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Verrucomicrobia*, and *Cyanobacteria*, respectively. Overall, 10.3 % of the total most important rhizobacteria belonged to *Bacillus* followed by *Candidatus* Koribacter and *C.* Solibacter (8.0% and 6.3%), *Burkholderia* (5.2%), *Acidobacterium* (4.1%), *Pseudomonas* (3.9%), *Streptomyces* (3.5%), *Bradyrhizobium* (2.8%) and *Enterobacter* (2.6%); while the endosphere was dominated by bacterial genus *Serratia* (42.3%), *Methylobacterium* (7.6%), *Yersinia* (5.4%), and *Burkholderia* (2.2%). It was emphasized that the most common eubacteria in conventional and sustainable tea soils were *Proteobacteria, Acidobacteria* and *Actinobacteria* (Chen et al., 2021), and soil bacteria were dominated by *Proteobacteria, Bacteroidetes, Acidobacteria* and *Actinobacteria* during all tea-picking seasons (Zhang et al., 2020). A close relationship was found between increased soil moisture and high amount of *Bacteroidetes* in thirty-year-old tea plantations (Arafat et al., 2017). Beta-diversity analysis showed that organic, non-polluted and conventional tea plantation had significant differences in bacterial community of soils, whereas composition analysis indicated that *Proteobacteria, Acidobacteria, Chloroflexi, Actinobacteria* and *Firmicutes* (Tan et al., 2019), and *Acidobacteria, Proteobacteria, Actinobacteria* and *Chloroflexi* (Ji et al., 2018) were the most abundant bacteria in all tea plantation under varied management and fertilization practices. Strains of the *Proteobacteria* and *Acidobacteria* phyla are of great importance in terms of soil fertility, cycling of essential nutrients and sustainable nutrient management. Zhang et al. (2020) reported that *Proteobacteria* increased with cow manure and decreased with urea fertilization, furthermore, organic manure could enrich *Pesudomonas* and *Flavobacterium* to involve in the nitrogen and other biochemical cycle in tea plantation. Recent studies have shown that long-term organic farming practices can improve soil pH, soil microbial community and biodiversity of tea soils, and increase abundance of soil microbes, which played an essential role in biochemical reactions in tea orchard soils (Tan et al., 2019; Çakmakçı, 2019d).

3. FUNGUS ISOLATION TEA ORCHARDS

Nath et al. (2015), in a study of the isolation and characterization of endophytic fungi from root, stem and leaves of tea shrubs from different tea gardens and evaluated their PGP activities and ability of plant growth. Among ten different endophytic fungi isolated, the highest IAA activity was observed in *A. niger* followed by *P. sclerotiorum*, while the highest GA_3 activity was observed in *F. oxysporum* followed by *P. chrysogenum*. In their research, *P. sclerotiorum* was found as the most effective phosphate solubilizer, *A. niger* as potassium and *P.*

sclerotiorum as the most effective zinc solubilizer, and it was reported that these fungal endophytes were found to be efficient in PGP activities. In another study, six different fungi were isolated from tea roots and *Trichoderma* strains inoculated into tea seedlings were found to be resistant to some common pathogens (Augusta et al., 2006). Earlier studies (Singh et al., 2008), a consortium of 16 *Glomus* and 4 *Acaulospora* species developed from natural tea rhizosphere and a consortium of 14 species of *Glomus*, 2 *Acaulospora* and 1 *Scutellospora* from cultivated tea rhizosphere soil, both of consortia were found to promote the growth of tea plants in nonsterilized acidic soil. *Eupenicillium parvum* fungus isolated from the tea rhizosphere, showing a high level of tolerance to desiccation, salinity, acidity, and aluminum toxicity, showed a high P-solubility activity when rock phosphate was used as a source of insoluble P. Among the seven phosphate-solubilizing fungi isolated from the tea agro-ecosystem soil, *Trichoderma asperellum* was the most efficient phosphate solubilizer, followed by *T. harzianum, T. viride, T. viride, A. niger, A. flavus* and *Penicillium funiculosum*, respectively (Das et al., 2013).

Thirteen fungi isolated from the soil adhering to the feeder roots of tea, identified as *A. niger*, *A. versicolor*, *Discosia* sp., *E. parvum*, *Gliocladium roseum*, *G. virens*, *G. viride*, *Penicillium auranteogrisium*, *P. decumbens*, *P. lanosum*, *P. purpurogenum*, *P. grisefulvum*, and *Trichoderma pseudokoningii*, respectively, were reported to be able to solubilise phosphate (Rahi et al., 2009). A fungus identified as *Discosia* sp. was selected for further studies, exhibiting multiple PGP properties such as phosphate solubilization, biosynthesis of IAA-like auxins, phtase and siderophore production, and significantly increased root length, shoot length and dry matter in maize, peas, and chickpeas. While the most dominant fungus isolated from the tea rhizosphere was *Glomus* species, *Gigaspora*, *Acaulospora* and *Scutellospora* species followed, and it was found that the inoculation of *Glomus mosseae* increased different growth parameters such as leaf number, branch number and height in the tea plant (Bhutia, 2014).

4. PGPR IMPROVED GROWTH, NUTRIENT UPTAKE AND QUALITY OF TEA

In acidic tea growing areas, N and P are generally low and this deficiency can be increased by adding PGPR. Previous studies clearly indicate the potential of inoculations of PGPR in different conditions or increase the growth and nutrient content of tea. As a matter of fact, more growth and yield increases were observed in tea with the inoculation of native *Azospirillum* and P-dissolving bacteria together (Nepolean et al., 2012), as well as with the combined applications of PGPR and the ¾ of recommended N and P fertilizers (Saikia et al., 2011). Although PGPR such as *Bacillus* and *Pseudomonas* play a key role in increasing the growth and improving the efficiency of nutrition of tea bushes, biological fertilizers alone cannot meet the nutritional needs of tea. It was emphasized that nitrogen fixers can provide 20-50 kg N/ha, and phosphate solvents can provide 30-50 kg P/ha, which tea can take instantly (Gebrewold, 2018). In addition, biological fertilizers are a cost-effective approach to minimize synthetic fertilizer inputs, reduce

N_2O emissions, and develop best management practices for plant nutrients in the soil (Xu et al., 2014a).

On the other hand, the increase in the continuous and incorrect use of nitrogen fertilizers in tea gardens leads to low nutrient use efficiency and intense N_2O emissions. Previous research has shown that applications of *B. amyloliquefaciens* (Wu et al., 2018), *T. viride* (Xu et al., 2014a) and *P. polymyxa* (Zhou et al., 2020b) could reduce N_2O emissions and simultaneously increase plant growth and tea yield. It has been emphasized that N_2O emissions can be reduced by 33.3%-71.8% with *T. viride* biofertilizer (Xu et al., 2014a), and by 36.5%-73.1% with *P. polymyxa* biological fertilizer in addition to urea (Zhou et al., 2020b).

Soil fertility can be described as the capability of the soil to make nutrients available to plants, and it is necessary to integrate organics, chemicals, and biological resources for a more efficient and economical production system (Gebrewold, 2018). The use of biological fertilizers, a cost-effective supplement to organic and chemical fertilizers in tea farming, is very limited. The enrichment of chemical, organic and biological nutrient sources by combining them increases the efficiency of plant nutrient use and is becoming increasingly important as an economical and promising approach for environmentally friendly sustainable agriculture (Çakmakçı, 2019a). Similarly, the integrated use of native N_2-fixing *A. brasilense*, P-solubilizing *P. putida*, and K-solubilizing *B. cepacia* and *P. putida* isolated from tea orchard soils, together with organic and inorganic sources, has been proposed as an integrated nutrient management approach to preserve soil health and increase tea yield (Bagyalakshmi et al., 2012a). By using wastewater from the sweet potato starch industry as a liquid carrier, *P. polymyxa* biofertilizer was produced, tea growth, yield and tea polyphenol levels increased after foliar application, and it was emphasized that the application is an environmentally friendly method to evaluate waste (Xu et al., 2014b). The fact that sweet potato starch wastewater used in biofertilizer production contains a large number of nutrients such as N, P, K, Fe, Mg and Zn that can be easily taken up by leaves, production of auxin and antibiotic-like metabolites by bacteria and induction of systemic resistance have been explained as the mechanisms of promoting tea growth of biological fertilizer (Xu et al., 2014b). Çakmakçı et al. (2014) reported that the development of microbial consortia using organic and inorganic carriers including tea waste, peat, leonardite, perlite, zeolite, vermiculite, and liquid may be a practical and effective method for biofertilization of tea plant. These results show that triple inoculation with P-solubilizing and N_2-fixing bacterial bio-formulations enhanced trunk diameter, plant height, shoot weight, fresh and dry leaf weight, leaf area, and SPAD chlorophyll content of leaves in tea saplings. Chakraborty et al. (2010) reported that inoculation of wheat with *S. marcescens* and their bio-formulations of sawdust, rice husk and tea waste resulted in a consistent increase in growth in terms of height, leaf and branches number, and dry mass of tea leaves.

Screening of rhizobacteria that show multiple plant growth-promoting (PGP) traits suggests that they have a better potential for field testing and applications in improving the growth of tea (Çakmakçı, 2016). As a matter of fact, inoculation with a quartet consortium of *Brevibacillus agri, Aneurinibacillus aneurinilyticus, Sporosarcina koreensis* and *B. megaterium* strains with PGP properties such as IAA, ammonia, ACC deaminase and P-solubilizing ability resulted in significant increases in root and shoot length, root and shoot weight and leaf number in tea plant (Dutta & Thakur, 2017). The strains *B. subtilis*, *B. megaterium*, and *P. corrugata* were inoculated in tea soil, its capability to colonize the rhizosphere and root system of tea plants and its capacity to develop four tea clones and suppress rhizospheric fungal population has been reported by Trivedi et al. (2005). In addition, under gnotobiotic condition by Kumar (2017), determined that inoculation with *P. fluorescens*, *P. aeruginosa*, *B. cereus*, *P. aeruginosa*, and *Pseudomonas* sp. increased number of branches, shoot height, root length and chlorophyll concentration. In addition, a nursery experiment conducted by Morang et al. (2012), showed that inoculation with *P. aeruginosa* enhanced growth properties measured in terms of number of new leaves and lateral branches, shoot height and root length, fresh and dry weight of shoot and root, and chlorophyll content in tea plants. Borah et al. (2019) reported that 41% of 129 strains isolated from the rhizosphere of tea bushes showed ACC deaminase activity and that potent isolates could improve plant growth parameters such as dry/fresh root weight and shoot of tea plants.

Rhizospheric and endophytic bacterial species, such as *Azospirillum* species, *B. subtilis, B. pumilus, B. megaterium, B. amyloliquefaciens, B. atrophaeus, B. cereus, B. pseudomycoides*, *Bacillus lentus, B.*

licheniformis, B. simplex, B. agri, Brevibacterium sediminis, B. cepacia, B. pyrrocinia, P. polymyxa, Paenibacillus macquariensis, Paenibacillus taichungensis, Paenibacillus alvei, P. validus, P. agglomerans, S. koreensis, P. fluorescens, P. aeruginosa, P. putida, Arthrobacter globiformis, A. citreus, A. faecalis, M. luteus, C. acidovorans, R. erythropolis, R. fauriae, and *S. marcescens* have proven to promote *C. sinensis* growth through inducing N_2-fixation, ammonia-, siderophore-, and IAA-production, and P-solubilization, and/or exerting biocontrol activity against pathogens (Chakraborty et al., 2006, 2011, 2012; Erturk et al., 2008; Morang et al., 2012; Nepolean et al., 2012; Nath et al., 2013; Varmazyari et al., 2014; Dutta et al., 2015; Dutta & Thakur, 2017; Çakmakçı et al., 2013, 2014, 2015, 2017b, 2017c, 2018; Purkayastha et al., 2018; Chopra et al., 2020).

By using King's B agar, nine *Pseudomonas* strains isolated from tea rhizosphere were screened for their PGP activities, and all were IAA and siderophores producers and P-solubilizer, four were able to utilize cellulase and capable of inhibiting growth of *R. solani*, and three were found to enhance total shoot and root biomass of tea seedlings (Mazumdar et al., 2007). Inoculation with especially *E. lignolyticus*, *Burkholderia* sp., *B. pseudomycoides* and *P. aeruginosa*, which have high activity in terms of PGP traits, could increase root biomass production in tea 4.3 times, shoot biomass production 3.1 times, root length 2.2 times and shoot length 1.6 times under greenhouse conditions (Dutta et al., 2015). In nursery and field conditions, by inoculation of *B. cereus* PM43, *P. aeruginosa* PM105 and *Pseudomonas* sp. PM112 isolated from tea plantation; in tea plants, growth parameters such as chlorophyll, number of new leaves, shoot height, root length, fresh and dry weight, as well as increases enzymes such as PAL, POD, and PPO have been reported (Morang et al., 2018). Trial results show that inoculation with newly N_2-fixing and P-solubilizing bacterial strain *B. pumilus* AR-22, *B. subtilis* AR-17, *B. pumilus* AR-14, *A. globiformis* AR-33 and *Lysobacter enzymogenes enzymogenes* AR-4 stimulated overall plant growth, including enzyme activity, chlorophyll content, shoot development and leaf yield of tea (Varmazyari et al., 2014). Recent studies also demonstrated inoculation with eleven N_2-fixing, P-solubilizing and phytohormones-producing *P. agglomerans* PA58, *B. subtilis* BS21, *R. fauriae* RF272, *Achromobacter xylosoxidans denitrificans* AXD47, *P. polymyxa* PP243, *B. atrophaeus* BA59, *A. citreus* AC484, *R. radiobacter* RR112, *B. pumilus* BP70, *Pseudomonas agarici* PA71, and *B. subtilis* BS76 increased the growth parameters such as number of lateral roots, root length, plant height, root fresh and dry weight, trunk diameter, leaf weight, and chlorophyll contents (Çakmakçı & Varmazyari, 2019). Recently, Lin et al. (2019) carried out an experiment on tea under field conditions to investigate the effect of *B. amyloliquefaciens* liquid fertilizer on the yield and quality of tea bushes as well as the fertility of soil at the plantation and found that bacterial biofertilizer significantly improved the microbial diversity and fertility parameters such as total and alkali N, available P and K, organic matters, and humic acid, and increased the yield, water extracts, and free amino acids of the tea. A perennial tea plant grown as a monoculture in the same soil for many years gradually impoverishes the soil in terms of nutrients. Research reveals that foliar inoculation of PGPR significantly increases tea yield, quality, and concentrations of polyphenols due to N and P nutrition (Xu et al., 2014b).

5. CO-INOCULATION OF MICROORGANISMS

Combined inoculation of microorganisms with multiple PGP traits provides more balanced nutrition for plants (Şahin et al., 2004) and could be an alternative to application with individual strains, possibly reflecting the different mechanisms of each strain in the consortium. Often combined applications of the microorganism give better results. More recently, Shang and Liu (2021) evaluated the possible effects of a consortium that included quadruple strains, *T. asperellum*, *P. taichungensis*, *B. cepacia*, and *B. cereus* on tea growth under pot and field conditions. Co-inoculation with multi-traits bacteria-based consortia developed the physical and chemical properties of soil and stability of aggregates, changed the rhizobacteria diversity, influenced the native rhizobacterial community, as well as increased the plant parameters such as bud density, stem diameter, root and shoot weight, leaf area and hundred-bud weight. They found that consortium significantly increased organic matter, total N, available P, available K and water-stable aggregates under field conditions. Although dependent on the parameters evaluated and inoculant formulations, co-inoculations of multi-specific bacteria can alter the microorganism's community and affect tea growth, quality, and yield (Çakmakçı et al., 2015).

The dual application of arbuscular mycorrhizal fungi (AMF, *Rhizophagus fasciculatus*) and PGPR (*B. pumilus*) in the tea plants improve in the growth of seedlings in terms of increase in height and number of leaves (Chakraborty et al., 2015). Bacterial formulations could change the tea plantation soil fertility, soil physical-chemical and biological properties and environment, enzyme activity, and stress tolerance. Among the four different bacterial multi-traits consortia developed from indigenous isolates, BF15 (*B. subtilis* 2/8+ *P. putida* 62/5+ *B. subtilis* 39/3) and BF14 (*B. megaterium* 55/1+ *P. putida* 53/5+ *P. macquariensis* 69/2) mixed bio-formulations were found to be the most effective in increasing the growth, yield and quality, and activities of enzymes such as GR, GST, POD, G6PD, 6PGD, ADH and DHSK of tea (Çakmakçı et al., 2017c). Since applications based on a single strain can lead to inconsistencies, the inclusion of different species, beneficial and compatible microbes with different mechanisms in the same microbial consortium will prevent failures and inconsistencies, as well as improve plant performance and increase effectiveness.

In the existing agricultural systems, mostly nutritional imbalances increase, productivity decreases, adverse conditions in ecosystems gradually increase and agroecosystems are adversely affected because of the combination of negative factors originating from agriculture and non-agriculture. For these reasons, effective use of biological components such as PGPR and fungi is required to integrate nutrient management strategies to obtain higher input use efficiency, to achieve the desired productivity by using all resources effectively, to ensure its sustainability, and to use integrated nutritional and protection strategies and cope with increasing chemical input costs. Thus, soil-based AMF consortium developed from natural tea rhizosphere and cultivated tea rhizosphere soil in previous studies was found to remarkably promote the growth of tea crops in non-sterilized acidic soil (Singh et al., 2008). With the co-inoculation of N_2-fixing *Azospirillum,* P-solubilizing bacteria and vesicular-arbuscular mycorrhiza (VAM) fungi, more growth and yield were observed in tea plants, and it was stated that biological fertilizers could be used to improve tea bushes, increase yield, and reduce the amount of fertilizer (Nepolean et al., 2012). Yang et al. (2021) conducted experiments in field conditions to determine the effect of biochar-based biofertilizer containing fungal species as well as bacterial species, determined that the consortia increased the yield and quality parameters of tea along with the improvements in the chemical and microbial properties of the soil.

There are several advantages with the combined use of AMF and P-solubilizing and N_2-fixing rhizosphere bacteria. Depending on the plant species, the nature of the microbial strains, their ability to metabolize nutrients, and the soil nutrient status, as a result of millions of years of interaction, associative, symbiotic, neutralist, or parasitic interactions have developed in and around the root as well as plant and microbe species. Ensuring the management and increasing the efficiency of such interactions is a promising sustainable approach that can provide resources related to plant health and productivity (Nadeem et al., 2014). These interactions lead to synergistic effects on plant growth between the plant, bacteria/fungus, and other microbes as long as each partner also benefits as a mutual relationship and consortium members positively influence each other for a long time. The fact that the applied PGPR and microbial consortium positively affect the mutual relations between plant and native microorganisms, interacts positively with each other, is a driving force for host development and the application environment is an attractive habitat for microbes increases the success of the consortium. In this respect, combinations of microorganisms of different types and characteristics, especially N_2-fixing and P-dissolving bacteria, should be carefully prepared by taking into account microbe interactions, in order for each microorganism entering the mixture to affect each other synergistically and to improve the effectiveness of biological consortiums (Şahin et al., 2004).

6. INOCULANTS REDUCE CHEMICAL FERTILIZATION

Previous studies have focused on the development of formulations that can improve plant productivity in field conditions and effectively reduce the need for chemical fertilizers, promote efficient interaction with the plant and ecological nature, and reduce production costs. The use of PGPR and others as a biofertilizer, which can produce the same crop yield and reduce chemical fertilization by half or even more, is advantageous for agricultural security and the development of sustainable agriculture, due to both the high costs of chemical fertilizers and their negative effects on the environment. PGPR inoculants can contribute to the improvement of yield components and increase nutrient use and agronomic efficiency by decreasing costs and pollution, once the need and use of chemical fertilizers can be reduced or eliminated if the inoculants are efficient (Souza et al., 2015). Tennakoon et al. (2019) evaluated the possible effects of N_2-fixing, P-solubilizing bacteria-based bio-fertilizers in dual strains combinations (*Azospirillum* sp. + *Rhodococcus* sp., *Azospirillum* sp. + *B. cereus* and *Azospirillum* sp. + *Microbacterium* sp.), the results showed that inoculations increased tea growth and yield, and 1/3 N and 1/2 P reduction from the recommended fertilizer dose.

Inoculation with multi-trait bacteria allowed half-rate inorganic fertilizer application and enhanced yield and quality. Despite the reduction of N by 25% and P by 50% by inoculation with native species of *Azospirillum* and P-solubilizing bacteria, increased yields were reported in tea (Saikia et al., 2011). In another study conducted under field conditions, the highest green tea leaf yield was obtained when the biological fertilizer consisting of *Mycorrhiza*, *A. brasilense* and phosphate-dissolving bacteria was supplemented with 62.5% or 75% of the recommended chemical fertilizer (Easwaran et al., 2003). Similarly, in a previous study, nutrient uptake and yield increase in tea were reported when *Azospirillum* and P-solubilizing inoculants along with the low level of inorganic fertilizer for tea (Easwaran et al., 2002). Also, the combination of inoculation of fungi and *B. megatherium* from tea rhizosphere and 50% of the recommended quantity of recommended chemical fertilizer showed improvements in soil exchangeable K, organic carbon, available phosphorus, microbial biomass carbon, cation exchange capacity, and similar yield performances were recorded with recommended fertilizers alone (de Silva et al., 2014). It was concluded that biological fertilizer in tea cultivation, when combined with chemical fertilizers, has the potential to reduce chemical fertilizer inputs.

7. RECENT TECHNIQUES FOR PGPR

Research has mostly focused on the isolation, detection, identification, and evaluation of beneficial microorganisms from the plant rhizosphere, and its examination by basic morphological, physiological and biochemical tests. For the detection and characterization of PGPR, molecular such as Amplified rDNA restriction analysis (ARDRA), Repetitive element PCR (rep-PCR), RISA, Randomly amplified polymorphic DNA (RAPD), Denaturing gradient gel electrophoresis (DGGE), Automated ribosomal intergenic spacer analysis (ARISA) and 16S rRNA gene pyrosequencing, and immunological techniques such as ELISA, Immuno-dot-blot assay, Immunofluorescence colony staining and Immunomagnetic separation is used (Agrawal et al., 2016). Molecular techniques such as denaturing gradient gel electrophoresis (DGGE) (Juan et al., 2012) and immunological techniques such as ELISA and Immuno-dot-blot assay (Chakraborty et al., 2006, 2010, 2013) have been used recently for the detection, diversity, sustainability, and characterization of PGPR in the tea rhizosphere.

Recently, the selection of plant waste and inert materials that can enhance product stability, shelf life and efficiency in the field, enrichment of fertilizers with different metabolites, the use of microbiologically enriched fertilizers, coating technologies developed to reduce fertilizer volume and cost, bio-encapsulation and carrier-based techniques and inorganic, organic and microorganism mixture consortia stand out as important steps (Çakmakçı, 2019e). PGPB-based inoculation technology and bacterial-impregnated fertilizer should be used by impregnating the fertilizers with microbes along with the appropriate fertilization levels, in order to achieve the maximum benefit in terms of saving fertilizer, increasing the efficiency of fertilizers and better growth. Adoption of approaches to increase product performance, especially the selection of local and effective strains for microbial fertilizers of the desired quality and stability, the development of technologies such as fermentation, encapsulation, coating, lyophilization, co-encapsulation, lyophilization, and impregnation, as well as the use of suitable coating materials, good preservatives, stimulants, and enriching compounds should be considered as important approaches (Çakmakçı, 2019a). Designing efficient microbial formulations, both by integrating microbial sources among themselves or with organic sources, and by combining three important organic, inorganic, and microbial sources in a single formula is a promising new research area in terms of nutrient use efficiency, plant growth, agricultural economics and sustainability.

Since one of the most important problems of today is the development and application of sustainable farming techniques, the investigation of the rhizospheric and endophytic bacterial community of tea plants with their unique characteristics should be widespread. Thus, progress will be made in the development of bio-formulas, which are an important component of integrated plant nutrient management for sustainable agriculture and contribute to the increase, accessibility and concentration of plant nutrients, and the protection of human, soil, environment, and plant health. Effective microbial formulations that have positive effects on the environment and plants and are compatible with mineral resources, integrated strategies to maintain crop production and soil fertility, as well as the combined use of biological, mineral, and organic resources are increasingly accepted as promising approaches.

Efficient PGP strains and new biotechnological approaches should first be developed in order to develop formulations with long shelf life, increased shelf life, broad-spectrum and consistent field performance (Aloo et al., 2020). Optimization of growing conditions, tolerance and high productivity. Mass production methods of effective, stable, safer, inexpensive, new, and multi-featured bio-formulations need to be developed. The careful isolation of the rhizosphere microbiota, the identification of active isolates in different conditions, the use of biotechnology, the elucidation of plant-microbe interactions, and the development of specific bio-formulas for certain conditions and particular plants are very important.

CONCLUSION

It is important to research beneficial bacteria in order to develop technologies to increase inoculum efficiency, which is one of the most important sustainable practices in agriculture, and to develop new and efficient inoculants for agriculture. Microorganisms are the main components of soil and plant health that improve and enrich the nutrient quality of the soil, make plant nutrients available to the plants at low cost, increase soil fertility and crop productivity, influence plant composition, productivity and sustainability, and supplement chemical fertilizers. The microbial community components and quality indicators of tea rhizosphere soil such as abundance, biodiversity, activity and stability ensure the stability and productivity of tea production ecosystems and contribute to soil health and agricultural sustainability with its capabilities such as nutrient acquisition, fixation, mobilization, decomposition, recycling, degradation and improvement in the soil.

Plant growth-promoting microorganisms can exert a beneficial effect upon plant growth and have been considered as one of the possible alternatives for inorganic fertilizer for promoting plant growth, yield and quality. Despite all this, there is a need for extensive research on the interaction of PGPR with other microorganisms in different stress environments that are not fully elucidated, and to develop new strategies to facilitate sustainable agriculture in stressed plants and soil conditions. Both basic and applied research is required to elucidate the yet unknown aspects of these microorganisms and to make PGPR an efficient technique in sustainable tea cultivation. The application of agriculturally new and beneficial bacteria to the soil system emerges to maintain sustainability and reduce production costs without harming the environment.

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CHAPTER 2

A SUSTAINABLE AND EFFECTIVE STRATEGY IN SALINITY STRESS MANAGEMENT: PGPB-PRIMING

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INTRODUCTION

The most critical challenge for agricultural production is to fulfill the nutrient demand of the World population that is growing at a rate of around 1.05% per year (World Bank, 2019). Various biotic and abiotic factors such as salinity, drought, heavy metals, extreme temperature and other contaminants are limited adaptation, growth and productivity of plants, therefore, they lead to significantly reduce crop yield and quality (EL Sabagh et al., 2020). Out of all abiotic stresses, salinity and drought due to changing climate are the most harmful ones (Pushpavalli et al., 2014; Daliakopoulos et al., 2016) and threaten agricultural productivity, food security and human health.

Salinity occupies a pivotal position among environmental stress factors disrupting agricultural production. According to the report of FAO (2015) on the status of the World's soil resources, salinity adversely affects over 60 million hectares or about 20% of the irrigated arable area in the world. Moreover, it is estimated to increase salt-affected areas up to 50% of total arable land by 2050 (Kumar et al., 2020a). It has been determined to increase as a result of different irrigation malpractices, the accumulation of generally Na^+ and Cl^- ions in the soil, and changing environmental conditions (Shi-Ying et al., 2018; Wei et al., 2021). High Na^+ accumulation restricts water conductance and aeration. Moreover, increasing salinization in soil adversely affects the microbial diversity and activity within and around the rhizosphere of plants. Salinity stress causes several physiological, morphological and molecular changes, which inhibits growth, development and productivity in plants (Kumar and Verma, 2018; Dirik et al., 2020).

Salinity adversely affects legume growth by disturbing nutrition balances, the interaction of hormones, specific ion toxicity and osmotic effects (He et al., 2015; Patil et al., 2016). Salinity stress also disturbs the quality and yield of legume grains (Manchanda and Garg, 2008). Increased chlorosis and necrosis of leaves due to salinity, which causes leaf senescence and reduces photosynthesis in grain legumes (Khan et al., 2017). Salinity stress during the early seedling stage strongly reduces germination characteristics and agronomic traits, thereby, it leads to high yield losses and reduces grain quality (Jha et al., 2019; Ceritoglu et al., 2020; Özyazıcı and Açıkbaş, 2021).

Seed priming, which is based on soaking seeds to water or a low osmotic potential of solution pre-sowing, has commonly been used to both improve seed germination, seedling growth and also protect the plants against environmental stress and pathogens (Kumar et al., 2020b; Singh et al., 2020). Researchers state that seed priming applications provide faster and more homogeneous germination and improve seedling growth by controlled water uptake, activating starch disruption and enzyme actions, ATP synthesis and antioxidant defense systems, thereby, causes increase stress tolerance to negative environmental conditions (Farooq et al., 2017; Sita and Kumar, 2020; Ceritoglu and Erman, 2020; Açıkbaş and Özyazıcı, 2021). So, seed priming is a sustainable, easily applicable and cost-effective way to mitigate the adverse effects of salinity stress (Johnson and Puthur, 2021; Sheteiwy et al., 2021). Many different seed priming techniques have been used such as hydro-priming, osmo-priming, halo-priming, nano-priming, thermo-priming, solid matrix priming and bio-priming (Hasanuzzaman and Fotopoulos, 2019). Out of these, bio-priming with PGPR has a major position due to superior growth promoting and protecting properties of bacterial strains including ACC deaminase activity, P-solubilizing, N fixation, IAA and siderophore production, phytohormones secreting. So, bio-priming with PGPR is considered to be a sustainable and effective management strategy for salinity stress.

1. SIGNALING MECHANISM AGAINST SALINITY AND MORPHOLOGICAL, PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR RESPONSES IN PLANTS

The acceptable limit of electrical conductivity leading to no damage to crops is 0.7 dS/m, while over 3.0 dS/m sharply affects the growth, development, yield and quality (Ayers and Westcot, 1985). The general signal mechanism of plants to salinity is primarily based on the perception of signals by molecular receptors on the surface and secondary messengers such as ROS and inositol phosphatase (Tanveer and Ahmed, 2020). The task of secondary messengers is to control gene expression via modulation of Ca^+. The Ca^+ has a pivotal role in first signaling at the beginning of salinity stress (Kurusu et al., 2015). Out of the signaling molecules, salicylic acid is particular attention due to its capacity to regulate different aspects of plant responses to salinity stress via extensive signaling cross-talk with other growth substances

(Jayakannan et al., 2015). There are several other genes controlling hormones and physiological and cellular activities.

Salt stress leads to higher concentrations of Na^+ accumulation in plant tissues, changes the K^+/Na^+ ratio and reduces nutrient and water uptake by plant roots. The high concentration of Na^+ in the root enables osmotic stress, reduces water potential and breaks the nutrient balance. Over the limit of Na^+ outside the plant cell adversely affects intracellular K^+ influx, thereby, the physiological processes are damaged since K^+ is an essential element for plant growth (Singh et al., 2015). Moreover, excess Na^+ inside the cell inhibits various processes including seed germination, seedling growth, flowering, and grain formation (Özkurt et al., 2018; Arif et al., 2020). Taibi et al. (2016) determined that Na^+ content of leaves *Phaseolus* sp. increased 5-7-fold compared with control plant's leaves, while the K^+ content decreased between 32-35% depending on salinity stress. Similarly, the Na^+ content of leaves *Cicer* sp. increased 3.2-fold compared with the control plant's leaves, While the Na^+/K^+ ratio increased to 2.24 in the non-saline condition from 0.31 in the saline condition (Abd-Allah et al., 2018). Ceritoglu et al. (2020) stated that the total biomass of chickpea cultivars decreased up to 12.5-54.2% depending on 100 mM NaCl treatment compared with control plants.

Salinity stress damages cell membrane stability resulting in increased permeability, which causes electrolyte leakage and its accumulation around tissues (Sandhya et al., 2010). Except for losing of turgor and low water potential, salinity restricts stomatal conductance, transpiration rate, decreases the photosynthetic pigments of the leaves, the leaf surface area and photosynthesis efficiency and causes leaf senescence (Alamri et al., 2020; Lofti et al., 2020; Pan et al., 2020). The inhibition of photosystem II activity, which is a pivotal site of the electron transport chain, occurs during salinity stress (Kalaji et al., 2011). Moreover, salt stress causes unrepairable damage to several metabolic functions such as chloroplasts structure, envelope and protrusions (Wungrampha et al., 2018).

Some cellular enzymes such as DNase, RNase and proteases are adversely affected by salinity (Ivanina et al., 2020). Also, salinity stress induces plant hormones such as ABA, JA, ethylene and causes increasing antioxidant responses including CAT, GR, SOD, APX and GPX (Moghaddam et al., 2020). Another biochemical change observed in plants under saline conditions is ion homeostasis and osmolytes accumulation such as proline, betaine and glycine (Kumar and Varma, 2018; Datir et al., 2020). Salinity also provides the accumulation of reactive oxygen species (ROS) such as superoxide radicals, singlet oxygen, and H_2O_2. The electron transport chain in the chloroplast and mitochondria are important sites of ROS production under saline conditions (Gill and Tuteja, 2010). In general, physiological, biochemical and molecular impacts of salinity stress in plants were schematized and summarized in Fig 1.

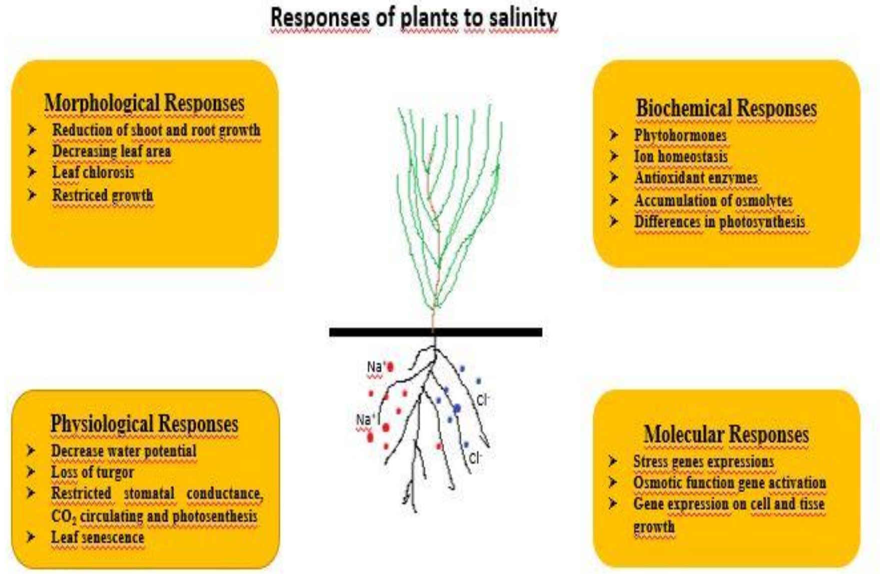

Fig 1. Morphological, physiological, biochemical and molecular responses of plants under saline conditions.

There are many management strategies to salinity stress such as breeding tolerant genotypes, biotechnology and functional genomics, using arbuscular mycorrhizal (AM) fungi, PGPB inoculation, exogenous hormones and osmoprotectants treatments, seed priming techniques and nutrient management (Farooq et al., 2017; Gupta et al., 2020; Iqbal et al., 2020). Among these management techniques, bio-priming with PGPB has a major position due to superior growth promoting and protecting properties of bacterial strains including ACC deaminase activity, P-solubilizing, N fixation, IAA and siderophore production, phytohormones secreting. So, bio-priming with PGPB is considered to be a sustainable and effective management strategy for salinity stress.

2. SUPERIORITIES OF PGPB-PRIMING TO OTHER SEED PRIMING TECHNIQUES

Although commonly used seed priming techniques provide faster seed germination, better seedling growth, higher crop yield and quality, and increasing tolerance to abiotic stress factors, bio-priming with PGPB can protect the seeds against pathogens and increase adaptation to different biotic stress factors (Delvi et al., 2013; Prasad et al., 2016). Moreover, PGPB-priming is not only a superior technique to other priming methods, but it is also more effective than pelleting and film coating practices in disease management (Muller and Berg, 2008). PGPB-priming provides higher carbohydrate reserves that fortify the plant to stay alive under low oxygen and waterlogged conditions compared with non-primed seeds (Ella et al., 2011). PGPR-priming leads to combat against pathogens due to their antagonistic impacts, thereby, it is also an eco-friendly and sustainable equivalent to chemical fungicides (Deshmuks et al., 2020). All of these properties lead to consider that PGPR-priming is an applicable and effective biological weapon for sustainable agriculture.

On the other hand, PGPB-priming directly contributes to plant growth and development by secreting compounds and solubilizing minerals (Sukanya et al., 2018). While other seed priming techniques affect the plants in several ways, PGPB-priming is able to involve a biofertilizer, bio-agent, bio-stimulant, or combination of some of them. Bio-priming with a P-solubilizing PGPR both causes faster and homogeneous germination and also provides to increase soluble P compounds in the rhizosphere due to excretion of organic anions, hydroxyl ions, protons, carbon dioxide, or phosphatase enzyme, therefore, they contribute to morphological, physiological and biochemical growth of plants (Glick, 2020). Similarly, diazotrophic bacterias, symbiotic or free livings, provide an obtainable nitrogen source that has a pivotal position for plants during different growth stages by biological nitrogen fixation (Mus et al., 2018). The stimulation of plant growth by siderophore producing PGPR occurs directly supplying iron for plant and/or removing iron from the environment of nearby phytopathogens thereby decreasing the competitiveness of phytopathogens (Glick, 2020).

Most of the physiological activities in plants are regulated by phytohormones such as auxin, gibberellin, cytokinin, ethylene, abscisic acid, jasmonic acid, salicylic acid and brassinosteroids. (Checker et al., 2018). Besides, some soil bacteria can change the synthesizing and/or modulate the level of some phytohormones. Phytohormones help to adjust plant growth and patterns under different environmental and developmental conditions (Xu et al., 2018). Out of the phytohormones, ethylene is involved in the various critical process including seed germination, tissue metamorphosis, root and shoot formation and growth, flowering, anthocyanin synthesis, fruit ripening and degreening, production of organic compounds, hydrolysis of storage products, leaf senescence, root nodulation, mycorrhizae-plant interaction, and response to stress factors (Vandenbussche and Straeten, 2018). In addition, Dubois et al. (2018) stated that the ethylene is involved dual-position under stress, in which it generally regulates a defense response in old leaves while it forms a growth response in young ones. In fact, promoting or inhibiting the impact of ethylene is depending on its production amount.

Due to inhibiting the impact of high secreting ethylene, bacterial strains which have ACC deaminase activity have a critical role in stress management. ACC (1-Aminocyclopropane-1-carboxylic acid) is a precursor of ethylene synthesis under stress conditions. The mission of the ACC deaminase enzyme in this process is to convert the ACC into ammonia and α-ketobutyrate (Fig 2), thereby, plant growth is improved under stress conditions via reducing ethylene inhibition (Orozco-Mosqueda et al., 2020). Also, reducing ACC can enable the level of ethylene inhibition of the IAA signaling process, thereby providing bacterial IAA to more effectively simplify plant growth and development.

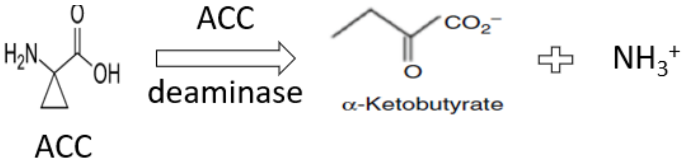

Fig 2. Chemical degradation of ACC into α-ketobutyrate and ammonia via catalyzing ACC deaminase enzyme. Due to ACC deaminase enzyme activity, both nitrogen and energy are provided for cells and tissues in addition to restricting stress ethylene, thereby, plant growth and development are stimulated under stress conditions.

In addition, ethylene has a pivotal role in nodule formation acting as an inhibitor of the nodulation process. Grobbelaar et al. (1971)

demonstrated that 0.4 ppm exogenous ethylene noteworthy inhibited nodule formation and nitrogen fixation in *Phaseolus vulgaris*. Many authors denoted that ethylene is involved in several stages of legume-bacteria symbiosis such as first bacteria-plant interaction, Nod factors, nodüle formation and development, senescence, and abscission (Csukasi et al., 2009; Patrick et al., 2009; Paço et al., 2020). So, Rhizobial bacterias which have ACC deaminase activity lead to increase nitrogen fixation capacity by blocking ACC and ethylene production under stress and non-stress conditions.

All these properties denote that PGPB-priming can be an effective strategy in terms of stress management and have a superior and sustainable role compared to other priming methods.

3. THE STUDIES ON ALLEVIATING IMPACTS OF PGPB-PRIMING TO SALINITY STRESS IN LEGUMES

Although salinity stress adversely affects the microbial communities and their functions, salinity-resistant strains can sustain vital functions at the same rate to a certain degree. Resistant strains are able to regulate the physiological process, improve plant health, sustain ecological functions, protect and promote plant growth under salinity stress (Ha-Tran et al., 2021). In fact, salinity-resistant strains can survive up to 30% of salt concentration and tolerate the adverse impacts of salinity stress by various mechanisms including the production of extracellular proteases, accumulation of compatible solutes for osmoregulation and activation of Na^+/H^+ antiporters (Kumar et al., 2020a).

Plant growth promoting rhizobacteria stimulates nutrient and water acquisition and increases the adaptation against stress conditions. It is considered that reducing ethylene level by ACC deaminase enzyme activity is the main reason for PGPR-induced stress management. Another major property of PGPR strains used for stress management is phytohormones production such as auxin, gibberellin, cytokinin and IAA. Many bacterial strains are involved in PGPB-priming applications in different grain legumes exposed to salinity stress in which various mechanisms of action have been determined by researchers (Table 1.)

Table 1. Action mechanism of PGPB inoculation against salinity stress in various grain legumes

Grain Legumes	Bacterial Strains	Action/Mechanism	References
Faba bean	*A. brasilense* *Rhizobium sp.*	Promotes root and shoot growth, nodulation and crop yield, reduces salinity stress	Hamaoui et al. (2001)
Mung bean	*Enterococcus mundtii* *Rhizobium* spp.	exhibits higher performance on macro- and micro-nutrient uptake, seed germination, plant growth and total biomass, chlorophyll content. Increases phosphatase and dehydrogenase enzyme activities	Kumawat et al. (2021)
	Pantoea sp. and *Enterococcus*	Increases tolerance to salinity stress by ACC deaminase activity and promotes plant growth	Panwar et al. (2016)
Soybean	*Trichoderma harzianum*	Improves root and shoot growth and leaf greenness, causes higher proline accumulation and catalase activity under salinity stress	Khomari et al. (2018)
	Pseudomonas putida H-2-3	Upgrades colonization of bacterial diversity in the rhizosphere, ACC deaminase enzyme activity, and protection to salinity stress	Barnawal et al. (2014)

Pea	*Acinetobacter bereziniae, Enterobacter ludwigii, Alcaligenes faecalis*	Modulates proline content, chlorophyll content, total soluble sugar, electrolyte leakage and antioxidant enzyme activity. Reduces H_2O_2 content under saline conditions	Sapre et al. (2021)
	Arthrobacter protophormiae	Enhances chlorophyll content in leaf, increases shoot growth and dry matter accumulation in plants exposed to salinity	Kang et al. (2014)
Lentil	*Rhizobium* spp., *A. brasilense*	Increases seedling tolerance against salinity stress under in-vitro condition	Sijilmassi et al. 2020
		Promotes morphological growth and grain yield	Darabi et al. (2016)
Black gram	*Bacillus* and *Pseudomonas* spp.	Improves photosynthesis and nutrient uptake, increased nodulation, total nitrogen and phosphorus under saline conditions	Nagaraju et al. (2020)
Chickpea	*A. brasilense*	Promotes root and shoot growth, nodulation and crop yield, reduces salinity stress	Hamaoui et al. (2001)
	P. *pseudoalcaligenes*, P. *putida*	Increases number of leaves, branches, lateral root formation, fresh and dry weight, chlorophyll content in PGPR treated plants under salinity stress	Patel et al. (2012)
Common bean	*P. extremorientalis, P. chlororaphis*	Stimulates root colonization, dry matter accumulation, root and shoot growth	Egamberdieva (2011)
	Azospirillum brasilense, Rhizobium sp.	Promotes root branching and secretion of nod-gene-inducing species, enables longer and more persistent exudation of flavonoids improves the expression of nod-genes and nodulation patterns	Dardanelli et al. (2008)

Inoculation with PGPR having ACC deaminase enzyme activity induces root tips and lateral root formation, root colonization and root surface area, therefore, it promotes water and nutrient uptake, postpones the senescence, repairs the cells and tissues and increases the survival time (Egamberdieva et al., 2017). Ilangumaran et al. (2021) pointed out that inoculation of soybean seeds with rhizobacteria (*Rhizobium* sp. and *Hydrogenophaga* sp.) which are isolated from root nodules of indigenous legumes do not only induce plant growth and grain yield, and also improves K^+/Na^+ ratio in the shoot, nitrogen assimilation and stress tolerance under salinity stress. Inhibition of nodulation in legumes under salinity stress is caused by high ethylene production (Gresshoff et al. 2009) and changes in auxin level in roots (Liu et al. 2015). Thus, ACC deaminase activity and phytohormone production have a vital role in stress management.

Kumari et al. (2018) primed the mung bean seeds with BHU B13-398 (*Pseudomonas aeruginosa*) and BHU M (*Bacillus subtilis*) and determined that BHU B13-398 and BHU M increased shoot and root length up to 32.26%, 84.60% and 13.38%, 61.94% compared to unprimed seeds, respectively. Ahmad et al. (2013) observed the effects of *Rhizobium* and *Pseudomonas* strains to improve salt stress tolerance in mung bean. According to the results, *Rhizobium* and *Pseudomonas* strain enhanced dry matter accumulation up to 1.9- and 1.4-fold, respectively while co-inoculation of them increased dry matter accumulation by 2.2-fold due to their synergistic effects on each other. Metwali et al. (2015) primed the faba bean seeds with PGPR involved in *Pseudomonas putida*, *P. fluorescens* and *Bacillus subtilis* species under saline and non-saline conditions. Germination characteristics and seedling growth were induced by PGPR-priming under both salinity and normal conditions. Han and Lee (2005) indicated that plant growth, mineral uptake and photosynthesis were stimulated and proline, MDA, GR and APX activities increased in PGPR (*Bradyrhizobium japonicum*) primed of salt-stressed soybean plants. Ahmad et al. (2011) stated that co-inoculation of Rhizobium and PGPR involving ACC deaminase activity enhances salt stress tolerance and nodulation in mung bean. Chaudhary and Sindhu (2015) denoted that PGPR-priming with *Mesorhizobium* strains containing ACC deaminase promoted plant growth, nodulation and salt stress tolerance of chickpea plants.

CONCLUSION

Bio-priming with PGPB has a major position due to superior growth promoting and protecting properties of bacterial strains including ACC deaminase activity, P-solubilizing, N fixation, IAA and siderophore production, phytohormones secreting. Particularly, due to inhibiting the impact of high secreting ethylene, bacterial strains which have ACC deaminase activity have a critical role in stress management. salinity-resistant strains can survive up to 30% of salt concentration and tolerate the adverse impacts of salinity stress by various mechanisms including the production of extracellular proteases, accumulation of compatible solutes for osmoregulation and activation of Na^+/H^+ antiporters. Moreover, plant growth promoting rhizobacteria stimulates nutrient and water acquisition and increases the adaptation against stress conditions.

As a result, PGPR-priming can be considered a sustainable and effective method against salinity stress.

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CHAPTER 3

INTERACTION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) WITH MEDICINAL AND ORNAMENTAL PLANTS

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INTRODUCTION

Today, food is one of the most important vital factors for human life. The dramatically increasing population around the world makes this need even more crucial. According to the research of the United Nations; it is predicted that the human population living in the world will reach 9 billion by 2050 and it is expected that there will be a continuous increase in food demand and various problems will arise to overcome to this demand (Wezel et al., 2014; Alaylar et al., 2020). It is also estimated food demand will exceed 70% more food than today (Godfray, 2010). At the same time, people are becoming aware that sustainable agricultural practices are fundamental to meet the future world's agricultural demands. Therefore, numereous research have been done in the last century to meet this need (Shiferaw et al., 2011; Kour et al., 2020; Rezaei-Chiyaneh et al., 2020; Santos et al., 2020). Especially, the Green Revolution has been thought as an alternative solution to solve food demand all over the world. The Green Revoloution was initially existed to second part of the twentieth century and it was based on the usage of chemical fertilizers, pesticides and herbicides. At the beginning, the Green Revolution considerably promoted to enhanced plant productivity and crop yields in agricultural areas. Hence, global agricultural perspective has widely altered because of producers's high product demand of their agricultural lands (Basu et al., 2020). As a result of this perspective; Green Revolution; overutilization and unconscious usage of chemical fertilizers, pesticides and herbicides have been caused hazardous effects on not only alteration of biological and physicochemical properties of fertile soil but also environmental health problems (Srivastava & Singh, 2017, Alaylar et al., 2020). Hence, there is require to find alternative solutions, ecofriendly adaptive agricultural approaches for balancing to nutrient in the soil structure for deteriorate agroecosystem.

The importance of improving product yield, soil health and nutrition for sustainable agricultural practices is clearly mentioned in the previous studies in the literature (Haggag et al., 2007; Timmusk et al., 2017; Ferreira et al., 2019; Oleńska et al., 2020). In this regard, soil microorganism based fertilizers are usualy preferred to instead of chemical fertilizers nowadays. Among these soil microorganisms; bacteria are commonly performing fundemantal role in sustainable agricultural approaches as eco-friendly and safety agricultural products from the time of understanding of their beneficial properties. These bacteria group are called as plant growth promoting rhizobacteria (PGPR). Furthermore; the data suggested that PGPR can be exploited improve to soil and plant health and promote plant growth without any deleterious effects (Pandey et al., 2019; Pereira et al., 2019; Khanghahi et al., 2021).

For this reason, it has been a novel focus on studing the application of PGPR for commercial approaches especially in the areas of the fertile soil in agricultural lands to increase the sustainable agricultural applications. Therefore, two main subtitle will be mention of potential PGPR strains relation with medicinal and ornamental plants from previos reports in the literature and what kind of plant growth properties

(PGPs) were identified their potential usage in sustainable agricultural approaches.

1. PLANT GROWTH RHIZOBACTERIA (PGPR)

Plant Growth Rhizobacteria (PGPR) term was initially defined in the last decades of 1900s by Kloepper and Schroth (Zaidi et al., 2015; Katiyar et al., 2016). According to common definition; PGPR are heterogeneous soil group bacteria which are situated in the rhizosphere at plant root surface and constant relation with plant roots to improve plant growth directly or indirectly (Joseph et al., 2007; Alaylar et al., 2019; Alaylar et al., 2020a; Alaylar et al., 2020b).

For instance; PGPR perform as biofertilizer, enhancing presence of nutrients via nitrogen fixation, and solubilization of plent of soil minerals, like zinc, phosphate and potassium *etc*. (Lopes et al., 2021). Besides, PGPR exploited siderophores attain to significant iron sources in the agricultural areas (Sumbul et al., 2020). Furthermore, PGPR act as biostimulants, affecting to the numerous phytohormones whether increasing abscisic acid, gibberellins, cytokinins production or decreasing of ethylene production as direct mechanism (Khan et al., 2020; Santos et al., 2020; On the other hand; also several plant growth promoting properties (PGBs) are known as bioprotectant or biocontrol mechanisms with their pivotal roles for enhancing to resistance against to plant pathogens, via rivalry for uptake nutrients, and induces systemic resistance (ISR), antagonism with indirect mechanisms.

All these direct and indirect mechanisms, as mentioned above have been focused on numerous previous studies in the literature (Bhattacharyya & Jha, 2012; Oleńska et al., 2020; Santos et al., 2020; Hamid et al., 2021). Therefore, summarized PGPR forms and their mechanism were given in Figure 1. Since that first understanding of PGPRs beneficial effects, research activities aimed at understanding how these bacteria demonstrated their beneficial effect have dramatically improved and many previous studies have been reported various type of soil bacteria (Santos et al., 2020).

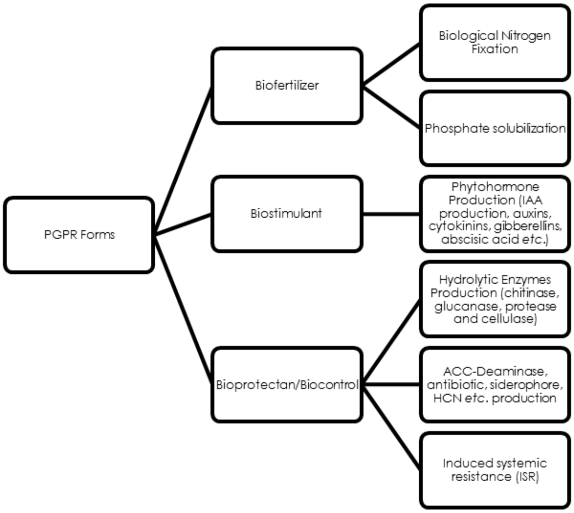

Figure 1: PGPR forms and their beneficial effects on plant growth

Up to now; PGPR term have been categorized into three various type of soil bacteria rely on their lifestyle and environmental conditions such as free living bacteria situated in the region of the plant root (rhizosphere), located in root surface (rhizoplane), and endopyhic bacteria which are existed in plant tissues (Saharan & Nehra, 2011;

Golinska et al., 2015; Ruzzi & Aroca, 2015). Although, any of the sole bacterial strain can be naturalize all of these three lifestyle according to the soil plant relation. (Ruzzi & Aroca, 2015). Much more prosperous literature regarding the isolation and identification of potential PGPR strains are available. According to the previous studies; numerous potential PGPR strains were containing strains members of the *Alcaligens*, *Arthobacter*, *Agrobacterium*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Serratia etc*. (Ruzzi & Aroca, 2015; Zaidi et al., 2015; Aeron et al., 2020). Hence, it is possible to isolate these potential strains from horticultural, medicinal and ornamental plants's rhizosphere, rhizoplane and tissues.

2. PGPR Relations with Medicinal Plants

Plants have been unique members of human life in terms of food and health since the beginning of life (Jamshidi-Kia et al., 2018). Especially, medicinal plants have been cornerstone of the health, food and economy not only the ancient times, but also today. Currently, it is well known that medicinal plants are among the vital components for traditional and modern medicine, which can be used in the treatment of many diseases (Abdel-Rahman et al., 2020; Egamberdieva et al., 2020). Recently, about 60-80% of the world's population still rely on these plants for the treatment of common diseases because of their beneficial seconder metabolites (Menghani et al., 2011). Nowadays, medicinal plants are gaining importance due to their characteristic benefits and valuable medicial effects, with their rapidly increasing usage around the world. In this perspective, most of drug companies have been focus on the discover new metabolites from various type of medicinal plants for use in international trade because of their high economic value. In this regard, the term "High value small crops" or “High-value minor crops” has been given to herbs, spices and medicinal and aromatic plants that promote to the agricultural production (Sher et al., 2014). According to the data obtained in 2010; the trade volume of medicinal plants and products of all countries around the world is around USD 60 billion. It is also estimated to reach USD 5 trillion by 2050 (Zahra et al., 2020). This situation is precisely indicated that require for medicinal plants has augmented in so many countries as conscious that natural products are non-toxic and without detrimental effects and can be provided at reasonable costs (Jamshidi-Kia et al., 2018; Rezaei-Chiyaneh et al., 2020). Besides, indiscriminate use of chemical fertilizers, herbicides were aimed to increase of medicinal plants and all other type of agricultural product yield. On the other hand, these strategies had demonstrated negative impacts on beneficial ingredients of medicinal plants. Meanwhile, chemical fertilizers have been caused detrimental effects on environment and plant, animal and human health. Due to these reasons, most of cosmeceutical, pharmaceutical, food producers or companies focus to fertilizers originate from sustainable and organic systems (Fonseca-Santos et al., 2015; Rezaei-Chiyaneh et al., 2020). Considering all known factors as mentioned above; it was a huge need to the chemical fertilizers were substituted by biofertilizers. In this regard, the usage of biofertilizers is an eco-friendly and novel application for sustainable organic farming (Patel et al., 2016). The knowledge on application of biofertilizers as PGPR have been accomplished like an eco-friendly method for increasing the yielding of agricultural products contaning medicinal plants.

Based on the literature review of the past decades, PGPR and medicinal plants relation were reported in plent of studies. It is clearly that PGPR are determined in the rhizosphere of many of medicial plants grown in various type of soils and environmental conditions all over the world. These microorganisms have positive effects on medicinal plants through different mechanisms such as protecting from biotic and abiotic stresses conditions, prohibition of diseases by producing valuable antagonistic compunds, production of plenty of hydrolytic enzymes, improving nutrient uptake via biological nitrogen fixation, phosphate solubilization, production of phytohormones including auxins, cytokinins and gibberellins, 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) enzyme production *etc*. thus promoting plant growth and resistance to exstreme environmental conditions, plant and diseases (Rana et al., 2020; Pushkaran et al., 2020).

Most PGPR have been reported to isolate from medicinal plants and exhibit at least one of the direct or indirect mechanisms as mentioned above. For instance; Thosar et al. (2005) were identified *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas* species from rhizosphere of *Withania somnifera* and they demonstrated to these group of bacteria had important role into enhance nutrient uptake in the rhizosphere. In the study conducted in 2008, *Azospirillum*, *Azotobacter* and *Pseudomonas* have been isolated from the rhizosphere of *Aloe vera*

Catharanthus roseus, Coleus forskohlii and *Ocimum sanctum* (Karthikeyan et al., 2008). Besides to isolation and identification of potential PGPR strains of medicinal plants, there are also numeorus studies have been conducted to previous PGPR strains which have been isolated different type of plants and they have been reported as how effect to these bacterial strains on medicinal plants under stress condions such as salt, water, drought and plant pathogens. For example; Lenin & Jayanthi 2012 were showed to how effects to mixture of PGPR strains such as *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Bacillus megaterium* and *Pseudomonas fluorescens* on germination and initial growth of *Catharanthus roseus*. The study clearly demonstrated that inoculation by PGPR strains considerably improved germination rate. Similar results have been found by Ghorbanpour & Hatami 2014 on *Salvia officinalis*. More information about identified PGPR strains from medicinal plants were given in Table 1.

Table 1: Plant growth promoting rhizobacteria (PGPR) interaction with various medicinal plants

Medicinal Plants	**PGPR Strains**	**References**
Aloe vera	*Azospirillum, Azotobacter, Pseudomonas*	(Karthikeyan et al., (2008)
Withania somnifera	*Azospirillum, Azotobacter, Bacillus, Pseudomonas*	Thosar et al., (2005)
Valeriana officinalis	*Bacillus, Pseudomonas, Erwinia, Xanthomonas, Agrobacterium*	(Ghodsalavi et al., (2013)

Origanum vulgare	*Stenotrophomonas*	Bafana & Lohiya, (2013)
Catharanthus roseus	*Azospirillum lipoferum, Azotobacter chroococcum, Bacillus megaterium*	Lenin & Jayanthi, (2012)
Ocimum sanctum	*Bacillus pumilus*	Murugappan et al., (2013)
Codonopsis pilosula	*Bacillus amyloliquefaciens* GB03	Zhao et al., (2016)
Plectranthus tenuiflorus	*Bacillus sp., Bacillus megaterium, Paenibacillus sp., Pseudomonas* sp.	El-Deeb et al., (2013)
Phyllanthus amarus	*Staphylococcus* sp. *Micrococcus* sp.	Kavita &Shanmugapriya,(2016)
Ocinum basilicum	*Bacillus* sp.	Kavita &Shanmugapriya,(2016)
Acalypha indica	*Bacillus sp.*	Kavita &Shanmugapriya,(2016)
Adhathoda vesica	*Serratia* sp. *Micrococcus* sp.	Kavita &Shanmugapriya,(2016)
Cadiospermum helicacabum	*Staphylococcus* sp. *Streptolococcus* sp. *Bacillus* sp.	Kavita &Shanmugapriya,(2016)
Spilanthes acmella	*Klepsiella* sp. *Enterobacter cloacae* *Pantoea* sp. *Burkholderia* sp.	Thakur et al., (2021)

Although, the microorganiss in the rhizosphere of medicinal plants are' not commonly discovered. Hence, further research is highly proposed to acquire the new percpectives to understanding of microorganism-medicinal plant relations. Therefore, potential PGPR strains to be isolated from medicinal plants will lead to an increase in product yield in the protection of these plants from pest and diseases and will contribute significantly to countries economically.

3. PGPR RELATIONS WITH ORNAMENTAL PLANTS APPLICATIONS

Ornamental plant term is used for commonly which have charming flowers attracted by people for private plant architecture applications such as increasing to human living environment conditions, agricultural farming, gardening *etc*. (Zheng et al., 2021). In this regards, ornamental plants were grown all over the world because of their beautifcation and decorative purposes. For this reason, ornamental plants have crucial role in landscape design (Vivek et al., 2021). It is well known that these plant groups have huge variation of beautiful shape of plants including cut flowers, flower bulbs, bedding plants, potted plants, outdoor and indoor plants *etc*. (Sajjad et al., 2017). In addition to the aesthetic beauty of ornamental plants to the environment, they also have a serious economic potential throughout the world.

Besides to the economic values of ornamental plants, medicinal properties of these plants are known from ancient times to the present, and they are widely used by people in both traditional medicine and modern medicine applications because of their beneficial bioactive compounds such as antioxidants, carotenoids essential oil, phenolic compounds, and numerous type of secondary metabolites (Saini et al., 2020). For instance, *Aloe vera, Calendula officinalis, Gardenia jasminoides, Nicotiana* sp., *Ixora* are well-known ornamental plants which have medicinal properties. These beneficial properties of ornamental plants clearly show that they are a very important touchstone in the pharmaceutical, food and industrial sectors (Saini et al., 2020).

In this regard, the rapid production of ornamental plants has been the focus of attention of producers because of their high demands and economic values all over the countries in the world (Zaidi et al., 2016; Sajjad et al., 2017). Considering the economic inputs of ornamental plants on countries, producers have been focused on the increase to ornamental plant growth, flower productions and quality criterieas like size of flowers and longevity (Zaidi et al., 2016). These situation is caused to use chemical fertilizers such as phosphorus (P), nitrogen (N) and potassium (K) by producers (Aeron et al., 2020). These elements have vital role in numeorus processes in plant nutrition and development. For instance; Nitrogen (N_2) is a primary element for all type of organisms on the earth and it is required for enzymes, proteins, nucleic acids and chlorophyll which have basic role in plant growth (Alaylar et al., 2019). P is one of the most crucial plant growth-restricting nutrient after N, P is plentiful element exist in the soil in not only organic form but also inorganic forms. But, many agricultural soils are still P-deficient in all over the world. Therefore, it is required to decrease P-deficiency in soils with P-fertilizers (Maurya et al., 2014). On the other hand, K is the third primary basic macronutrient and most plentiful absorbed cation that demontrates a pivotal factor in the plant growth and development (Meena et al., 2014; Aeron et al., 2020).

Due to the beneficial effects of these nutrients, many nitrogen, phosphate and potassium-based chemical fertilizers have been used for the development of many plants to increase plant growth and crop yield. The extensive and unconscious application of chemical based fertilizers to intensify crop cultivation is caused to seriously impact influence on arable soils (Egamberdieva, 2007). Moreover; when the producers and researchers have focused on the sustainable negative effects of the chemical fertilizers on plants are containing soil productivity, soil flora product yield are the main concerns of producers. Hence, as an alternative approach, microorganisms are significant role in with their beneficial properties provide to the circulation of plant nutrients and decrease to chemical fertilizers. As it is known previous literarute studies plant growth promoting bacteria have been profitable effects on plant nutrition by improving N and P uptake by the plants and these bacteria group called as PGPR because of their fruitful feautures on the biofertilization of crops. Up to date, many researches have been identified various type of PGPR strains which are positive effects on agricultural products (Table 2) such as agricultural plants which are including medicinal and ornamental plants. However, it is restricted studies about ornamental plants relations with PGPR. Therefore, it is required to fill this gap with more studies about ornamental plant-PGPR relations. Besides, there are a few researches about ornamental plants-PGPR relations to understanding of PGPR how effect to improve nutrient uptake, production of phytohormones antagonism effects against to plant pathogens. For example, Zaidi et al. (2016) have been summarized the effects of phosphate solubilizing bacteria group as PGPR strains how effects to various type of ornamental plants such as *Gladiolus grandifloras*, *Gerbera jamesoniiis*, *Petunia hybrid*, *Rosa damascene* and *Helianthus annuus etc*. In another study were conducted by Menéndez et al. (2016). They were reported *Rhizobium* sp. as a PGPR strain which had phosphate solubilization, siderophores and IAA production capabilities. Hence they tried *Rhizobium* sp. on *Dianthus caryophyllus* how to effect of this plant growth. The results demonstrated that effective root colonization and an enhancement to early stages of plant development. Park and co-workers (2017) were inoculated *Bacillus licheniformis* MH48 to ornamental plant *Camellia japonica* for understanding of how effect to nutrient uptake in seedlings of this ornamental plant. According to their report, *B. licheniformis* can be exploited as a potential PGPR because of its beneficial roles in the root development, nutrient uptake and mitigate salt stress in *Camellia japonica* seedlings grown in high salinity coastal land in South Korea.

Prisa (2019) was reported benefical microorganisms for germination and root growth in *Kalanchoe daigremontiana*. In the study of Nordstedt et al., (2020) also reported *Pseudomonas poae* 29G9 and *Pseudomonas fluorescens* 90F12-2 enhanced the quality of production *Petunia* × *hybrida*, *Impatiens walleriana*, and *Viola* × *wittrockiana* under adverse environmental conditions such as drought. Prisa & Benati (2021) note that various type of PGPR strains effects on ornamental bulbous plants such as Freesia, Iris, Narcissus and Tulip. The detailed information regarding effective PGPR strain and their mode of actions in the literature is given in Table 3.

As mentioned above, investigations into the interaction between ornamental plants and PGPR strains have demonstrated positive effects to ornamental plant development with various PGP mechanisms. Till date, when we evaluated to previous studies about ornamental plant-PGPR strains are still restricted. Therefore, it is still required to identified more bacterial strains to filled to this gap and understanding of their PGPs mechanism will have been important for the sustainable economic, healthy ornamental plant production.

Table 2: PGPR relations with various important agricultural products

PGPR	**Crop**	**Mode of Action**	**Reference**
Pseudomonas cepacia	Sunflower	Antibiotics production against to soil-borne pathogens	McLoughlin et al., (1992)
Alcaligenes piechaudii	Lettuce	Indole-3-acetic acid (IAA) production	Barazani & Friedman, (1999)
Pseudomonas fluorescens	Cucumber, Pepper	Antibiotics production against to soil-borne pathogens	Lee et al., (2003)
Herbaspirillum spp.	Rice	Nitrogen-fixing activity	Kennedy et al., (2004)
Bacillus polymyxa BcP26	Maize	Growth and nutrient uptake of in nutrient deficient	Egamberdieva, (2007)
Rhizobium leguminosarum	Carrot	Improved root length, dry matter of shoots and roots	Flores-Félix et al., (2013)
Azospirillum amazonense	Rice	Indole-3-acetic acid (IAA) production	Araújo et al., (2013)

Okibacterium sp. *Pseudomonas* sp.	Sugarcane	Phosphate solubilization	Pereira et al., (2019)
Arthrobacter sp.	Sugarcane	Nitrogen-fixing activity	Pereira et al., (2019)
Mesorhizobium ciceri Ca181	Chickpea	Phosphate solubilization	Yadav et al., (2021)
Bacillus subtilis MF497446 *Pseudomonas koreensis* MG209738	Maize	Siderophore production	Ghazy & El-Nahrawy, (2021)

Table 3: PGPR relations with various important ornamental plants

Ornamental Plants	**PGPR Strains**	**Mode of Action**	**References**
Petunia hybrid	*Bacillus polymxa*	Phosphate solubilization	Hoda & Mona, (2014)
Rosa damascena	*Acinetobacter* sp., Acetobacter sp., *Bacillus* sp., Micrococcus sp., *Planococcus* sp.	Phosphate solubiliziation, ACC deaminase activity, siderophore production, IAA production	El-Deeb et al., (2012)
Tagets erecta	*Paenibacillus polymyxa*	Phosphate solubilization	Ramlakshmi & Bharathiraja, (2015)
Petunia × hybrida	*Pseudomonas poae* 29G9, *Pseudomonas fluorescens* 90F12-2	Improved quality of the plant under adverse environmental conditions	Nordstedt et al., (2020**)**
Rosa damascena	*Pseudomonas fluorescens*	Improved shoot length	Tariq et al., (2016)

Impatiens walleriana	*Pseudomonas poae* 29G9, *Pseudomonas fluorescens* 90F12-2	Improved quality of the plant under adverse environmental conditions	Nordstedt et al., (2020)
Viola × *wittrockiana*	*Pseudomonas poae* 29G9, *Pseudomonas fluorescens* 90F12-2	Improved quality of the plant under adverse environmental conditions	Nordstedt et al., (2020)
Camellia japonica	*Bacillus licheniformis MH48*	Improved the nutrient uptake of plant	Park et al., (2017)

CONCLUSION

This chapter was aimed to summarize the current knowledge on PGPR relation with medicinal and ornamental plants. As it is known, medicinal and ornamental plants have a pivotal role in the many sectors which are related with medicine, foodstuffs, industry and the environment thanks to their economic value and beneficial compunds. Because of these economic values, many medicinal and ornamental plant producers have aimed to increase the production of these plants by using chemical fertilizers in the last past decades. Hence, excessive usage of this chemical fertilizers caused to deleterious effects on not only soil and plant interaction but also human health. Recently, we have witnessed plenty of advances due to the dissover of PGPR strains as novel approaches for safety, economic sustainable agricultural aplications which are called biofertilizers. Plenty of PGPR strains have beneficial effects on medicinal and ornamental plants via direct and indirect mechanisms for plant growth and development, nutrient uptake, disease resistance, yield and quality of medicinal and ornamental plant compounds. Currently, interest in the studies of relation of ornamental and medicinal plant with PGPR interactin are increasing day by day. On the other hand, it is needed to future studies to better understand the mode of action of rhizospheric bacteria and their benefical effects in the improved production quality of ornamental and medicinal plants by identifying more PGPR strains for sustainable eco-friendly approaches.

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CHAPTER 4

THE EFFECTS OF MACRO AND MICRONUTRIENT ELEMENTS AND RHIZOBIUM BACTERIA INOCULATION ON NODULATION, YIELD AND SOME YIELD TRAITS IN LEGUMES

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INTRODUCTION

These days when we are experiencing the effects of the global climate crisis intensely, the importance of sustainable agriculture is once again seen before us. In order for agriculture to be sustainable, we need to know the structures of plants as well as the applicability of the right agricultural techniques and make the most of them. In this context, the contribution of leguminous plants to agriculture should be considered. In order to benefit from the extraordinary abilities of these plants, it is necessary to provide suitable conditions.

Most of the 78% nitrogen in the atmosphere is composed of gaseous nitrogen molecules, and other living things, except diazotrophs, cannot benefit from this nitrogen. Biologically, all nitrogen-fixing prokaryotic organisms are bacteria. These are free or symbiotic as they live. Legumes have a special importance among diazotrophs. Approximately 70% of the nitrogen added to the earth in a year is provided by biological nitrogen fixation, and most of this is through leguminous plants. Leguminous plants and Rhizobium bacteria do this in the tubers called nodules in their roots (Togay et.al., 2019; EL Sabagh et al., 2020). This symbiotic relationship between the bacteria and the plant takes place in the nodule tissue, which is a specialized structure in the root tissue of the plant. Nodule formation involves a complex set of signaling relationships between plants and bacteria, starting with the synthesis of flavonoids secreted by plant roots (Yüzbaşıoğlu, 2021).

Many factors affect nodule formation and nitrogen fixation in legumes. The structure of the soil and its nutrient content is at the beginning of these. While most of the macro and micronutrients affect the nodulation positively, the deficiency or excess of some elements can cause negative effects. This interaction is usually related to the rhizobium bacteria and the way the nutrient is found in the soil or the way it is applied to the soil.

In this review, studies on macro and micronutrient applications and rhizobium bacteria inoculation in grain legume plants in the last two decades were investigated. The effects of nutrients and inoculation on nodule formation, yield and some yield elements were investigated.

1. MACRONUTRIENTS - RHIZOBIUM RELATIONSHIP

Biological nitrogen fixation, a key source of N for farmers using little fertilizer, constitutes one of the potential solutions and plays a key role in sustainable grain legumes production. Major benefits of the legume-rhizobia symbiotic interaction are diminished nitrogen fertilizer requirements and improving plant growth and health. Various studies have shown that Rhizobium inoculation, macroelements have the potential to improve legumes growth, seed yield, nitrogen fixation and also nutrient uptake of legumes. The impact of rhizobia inoculation in improving nitrogen fixation in legumes is widely documented (Bejandi et al., 2011; Elkoca et al., 2007; Ogutcu et al., 2008; Nyoki and Ndakidemi, 2014). For example, a study by Bejandi et al. (2011) and Soysal and Erman (2020) reported that seed inoculation with *Rhizobium cicerea* produced significantly highest nodule number, nodule fresh weight, nodule dry weight and active nodule per plant than control (Bejandi et al., 2011). Favorable effects of inoculation with *Rhizobium* spp. and significantly increase in nodulation and yield of legume crops have been reported by many researchers (Elkoca et al., 2007). Paudyal and Gupta (2018), Rhizobium inoculation technology for legume crop was evaluated in several field experiments comparing with 80 kg/ha urea application. The inoculation and urea application trial showed almost similar biomass accumulation, nodule number and nodule dry weight compared to un-inoculated control. Adeyeye et al. (2017) noticed that the use of fertilizers provided sufficient nitrogen which played a role in antagonistic effect for the rhizobial population to form the nodules and nitrogen fixation. It has been reported that the nitrogen application reduced the nodule number and mass of the *Glycine max*, *Phaseolus vulgaris* and *Pisum sativum* (Aslam et al., 2000; Muniz et al., 2017). Studies reporting that inoculation with rhizobia in field experiments rarely increased the yield of beans and gave no significant positive effects in nodulation and biomass production (Hungria and Vargas, 2000; Chekanai et al., 2018). However, there are also reports of successful use of rhizobia inoculation as an alternative nitrogen source in bean production. For example, Beshir et al. (2015) evaluated eight cultivars in a factorial combination of three nitrogen treatments (0 and 100 kg N ha−1 and *R. etli*) and found that both rhizobia and nitrogen increased the total yield of snap beans by 18 and 42%, respectively. Kacar et al. (2004), in their study to determine the effect of bacterial inoculation and different nitrogen doses on yield and yield components in some dry bean cultivars (*Phaseolus vulgaris* L.) in Bursa conditions, it was found that grafting had no effect on the properties examined on the cultivars and that the increase in fertilizer doses increased yield and yield components. reported increases. Reinprecht et al. (2020) reported in a study investigating the effect of nitrogen application on nitrogen fixation in beans; Bean genotypes differed for all analyzed traits, and the level of nitrogen significantly affected most of the traits, including %N dfa and yield in all three years. In contrast, the application of rhizobia significantly affected only a few traits, and the effect was inconsistent among the years. Nitrogen application reduced symbiotic nitrogen fixation (SNF) to various degrees in different bean genotypes. This variation suggests that SNF in the common bean can be improved through breeding and selection for the ability of bean genotypes to fix nitrogen in the presence of reduced fertilizer levels. Erman et al. (2009a), in their study investigating the effects of nitrogen doses (0, 20, 40 and 60 Kg/ha) and Rhizobium bacteria inoculation (inoculated and uninoculated) in peas, reported that the number of nodules increased up to 20 kg/ha nitrogen application in inoculated plants. They stated that as the amount of nitrogen dose increased, the number of nodules decreased. Otieno et al. (2009) studied the effects of farm manure, bacterial inoculation and nitrogen fertilizer on nodule formation and yield in edible legumes. They reported that nitrogen fertilizer reduced the number of nodules in some legume species, Rhizobium inoculation increased the number of nodules and the amount of nodule dry matter, but this had no effect on plant growth and fresh yield. Wheras, DAP, TSP, Urea and *Rhizobium Ciceri* bacteria were applied to chickpea cultivars in Diyarbakır in 2019, and it was reported that the highest number of nodules was obtained from DAP during the flowering period, followed by TSP fertilizer. It was stated that Rhizobium application alone gave the lowest values in both nodule number and yield (Eker, 2019).

It has been reported that legumes inoculated with rhizobium species and supplemented with P and K, respond differently in the growth, yield and nitrogen fixation (Yanni et al., 2001). A high phosphorus supply is needed for nodulation. When legumes dependent on symbiotic nitrogen receive an inadequate supply of phosphorus, they may therefore also suffer from nitrogen deficiency (Weisany et. al., 2013; Soysal and Erman, 2020).

The requirements of host plants for optimal growth and symbiotic dinitrogen fixation processes for P have been assessed by determination of nodule development and functioning (Almeida et al., 2000; Tsvetkova and Georgiev, 2003). The combined inoculation of Rhizobium and phosphorus has increased nodulation, plant height, seed protein and yield parameters in chickpea (Khurana et al., 2000; Namvar et al., 2011). Phosphorus is needed in relatively large amounts by legumes for growth and has been reported to promote legumes growth and yield, nodule number and nodule mass in different legumes. Erman et al. (2009b) stated that phosphorus applications and rhizobium bacteria inoculated significantly increased the number of nodules in peas, yield and yield characteristics statistically. Mohammed (2018) applied two doses of phosphorus (0 and 82 kg/ha) and two nitrogen (0 and 27 kg/ha) to peanuts, *Rhizobium* inoculated and uninoculated, on soils with low fertility. In their study, they reported that rhizobium inoculated phosphorus and nitrogen applications increased soil organic matter content, total nitrogen and available phosphorus amount.

Potassium influences the water economy and crop growth through its effects on water uptake, root growth, maintenance of turgor, transpiration and stomatal regulation (Mfilinge et.al 2014). Kurdali et al. (2002) studied the effects of different rates of K fertilizer on nodulation, dry matter production, and N_2 fixation by chickpea and faba bean subjected to different soil moisture levels at the beginning of the flower bud initiation stage. They found that plant species differed in their response to K fertilizer as a means of enhancing growth and overcoming the stress conditions. The higher level of K fertilizer increased both dry matter production and total N_2 fixed in faba bean, but did not have any impact on chickpea. Erman et al. (2012), statistical analysis of the study they conducted to determine the effect of potassium application on growth, yield and nodulation in different chickpea cultivars in Van conditions revealed that there were significant differences between years, between cultivars and between potassium applications, and that potassium application in the areas where chickpea cultivation is carried out in the region, yield, nodulation and quality. They reported that it has positive effects on the plant and that it would be beneficial for the producers to make potassium fertilization in addition to nitrogen and phosphorus fertilization. Soysal (2021), He investigated the effects of different doses of phosphorus applications in chickpea on yield and yield components of plants in Siirt province conditions. He reported that there are differences in phosphorus applications in the study. As a result, it was reported that 60 kg ha^{-1} P_2O_5 application had a significant effect on the yield in plants compared to the control application.

Sulfur deficiency decreases plant growth, photosynthesis, and seed yield in both legumes and non-legumes. In nodulated legumes, sulfur supply is positively linked to symbiotic nitrogen fixation (SNF) and sulfur starvation causes three additional major effects: decrease of nodulation, inhibition of SNF, and slowing down of nodule metabolism. These effects are due, at least in part, to the impairment of nitrogenase biosynthesis and activity, the accumulation of nitrogen-rich amino acids, and the decline in leghemoglobin, ferredoxin, ATP, and glucose in nodules. During the last decade, some major advances have been made about the uptake and metabolism of sulfur in nodules. These include the identification of the sulfate transporter SST1 in the symbiosome membrane, the finding that glutathione produced in the bacteroids and host cells is essential for nodule activity, and the demonstration that ulfur assimilation in the whole plant is reprogrammed during symbiosis (Becana et.al., 2018) nodulated legumes have a high demand for sulfur and SNF is more sensitive to sulfur deficiency than is nitrate uptake (Zhao et al., 1999; Varin et al., 2010). Not surprisingly then, legumes with a high sulfur supply show greater rates of N_2 fixation and, conversely, legumes grown on sulfur-poor soils have lower nitrogenase activity and readily respond to sulfur fertilizers by increasing yield and nitrogen content (Scherer, 2008). Barmaki and Samandar (2017) examined the yield and oil content in the study where they applied four different doses of sulfur fertilizer (zero, 200, 400,800 kg/ha), *Rhizobium* and *Thiobacillus* bacteria to the soybean plant. According to the results, it seems that using *Thiobacillus* & *Rhizobium* as PGPR with 800 kg ha-1 sulfur is advisable to improve soybean yield and oil production. Raj et al. (2017) reported that the seed inoculation with Rhizobium showed some good results increasing numbers of nodules and uptake of nutrients due to inoculation. Significant effects were observed in plants growth attributes due to the presence of phosphorus and uptake of phosphorus increased due to the presence of sulfur 20 kg/ ha ultimately resulting in good yield. However, plant heights (66.00cm), number of branches plant (4.82), number of nodules plant (5.83), number of grains pod (12.56), and grain yield (12.39 q/ha) were found significantly affected by the application of Rhizobium inoculation, application of 45 kg/ha phosphorus through DAP and 20 kg/ha sulfur through Gypsum ha. Mekuria et. al. (2019), investigated the effects of three different sulfur doses (0,20, and 40 kg/ha) and rhizobium inoculation (un-inoculated and inoculated) on nodulation, nutrient uptake and yield in lentils. They reported that the interaction of sulfur fertilization and Rhizobium inoculant were significant on days to flowering, number of nodules /plant, nodule dry weight/ plant, number of seeds /pod, aboveground dry biomass, seed yield, seed S uptake, haulm S uptake, total S uptake, sulfur agronomic and recovery efficiency as well as sulfur harvest index. The result indicated that S application has shown a tendency to be relatively more important in influencing the number of nodules per plant when seeds are not inoculated. The positive effect of Rhizobium inoculation and S may have created favorable soil conditions for the growth and development of nitrogen-fixing bacteria and promote the utilization of high quantities of nutrients through their well-developed root system for better nodules (Scherer, 2006). Togay et al. (2008a) reported that sulfur, phosphorus and *Rhizobium* bacteria inoculation had positive effects on yield, yield elements and nutrient uptake in chickpea calcareous soils. Sulfur fertilization and inoculation also significantly increased, number of nodules per plant, fresh weight and volume of nodules with an optimum rate of S in Faba bean (Kiros et. al. 2007). Tripathi et al.(2012),. applied different *Rhizobium* strains (Uninoculated, Tal 442 and MO 5) and sulfur doses (15, 30 and 45 kg/ha) to mungbean cultivars (SML-668, Pusa Vishal, and HUM-1). They reported that rhizobium strains, at par among themselves, and significantly superior over uninoculated. Cultivar HUM-1 and application of 45 kg S ha-1 recorded higher plant height, primary branches, green trifoliates, leaf area index, dry matter accumulation, nodule numbers and nodule dry weight, increased days to maturity, number of pod and higher grain and straw yield as compared to cultivars Pusa Vishal and SML-668, and S application at 15 and 30 kg ha-1, respectively. Nodule number was highest in HUM-1 × MO 5 and application of 45 kg S ha-1 in Pusa Vishal and HUM-1.

In soils with calcium deficiency, the amount of bacteria forming nodules decreases. Therefore, nitrogen fixation by leguminous plants is adversely affected (Kacar and Katkat, 2007). Calcium is known to act as a key intracellular messenger in the perception of symbiotic signals by both the host plant and the microbial partner. Regulation of intracellular free Ca^{2+}concentration, which is a fundamental prerequisite for any Ca^{2+-}based signaling system, is accomplished by complex mechanisms including Ca^{2+} binding proteins acting as Ca^{2+} buffers (Moscatiello et al.,2015).

2. MICRONUTRIENTS- RHIZOBIUM RELATIONSHIP

More recent studies indicate that this micronutrient is also essential for symbiosome development and bacteroid maturation and early events in plant-bacteria signaling (Redondo-Nieto et. al., 2003). Carpena et. al. (2000) suggested that a specific Boron- Calcium relationship mediated mobilization of Bor from old to new growing tissues in nodulated B-deficient pea plants and a higher requirement of B for nodules than for other plant tissues. Additionally, balanced Bor-Calcium nutrition has been reported to increase salt tolerance in the Rhizobium -legume symbiosis by ameliorating nodule development (El-Hamdaoui et. al., 2003). B and Ca are the nutrients with a major effect on legume symbiosis. Both nodulation and nitrogen fixation depends on B and Ca2+, with calcium more necessary for early symbiotic events and B for nodule maturation (Weisany et. al., 2013). Redondo-Nieto et al. (2003), who investigated the relationship between the legume Rhizobium in nitrogen fixation of boron and calcium, reported that the development of the symbiosis depends of the concentration of Bor and Calcium and that both nutrients are essential for nodule structure and function. Boron is required in the nodule and the meristematic system of the plant and the vascular bundles, and nodules cannot function without boron (Özdemir, 2002).

Molybdenum has positive impacts on growth, yield and nodulation in pulses (Lalita and Kumar,2017). When plants are cultivated under Mo deficient environment, the growth of plants becomes restricted due to phenotypes. These phenotypes are usually associated with reduced activity of molybdoenzymes that are involved in the primary nitrogen assimilation enzymes such as nitrate reductase (NR) and the nitrogen-fixing enzyme nitrogenase. That's why the role of Mo is considered positive on growth, yield, nodule forming, N content of roots and foliage in legume crops (Togay et al., 2008b). These researchers reported that the highest nodule number in lentils was obtained from 60 kg P ha^{-1} and 6 g/kg seed Mo applications, and the lowest values were obtained from control plots. Oguz (2004) in the study related to different Mo applications in chickpea reported that the highest nodules/plant was obtained from 6 g/kg seed Mo application. Aslam et. al. (2020) were 2 levels of *Rhizobium* inoculations (with and without rhizobia) and 5 levels of Mo (0, 3, 4, 5 and 6 g kg-1 seed) applied *V. radiata*. They reported that at maturity plant height, pod bearing branches/plant, nodules and pods/plant, seeds/pod, pod length, 1000-grain weight, seed yield and protein were significantly increased, where Mo (4 g/kg) + *R. phaseoli* was applied as compared to control. In addition, supplying of Mo may alsp facilitate nodulation, nitrogen uptake and seed protein formation in legumes (Bejandi et al., 2011). Paudyal et al. (2007) reported a higher number of nodules formation when inoculated with Rhizobium in the presence of Mo. Similarly, Hale et al., (2001) also reported a synergistic influence of Mo and nodule formation. Lalita and Kumar (2017) reported that positive effect of selected micronutrients (Mo and Fe) with *Rhizobium* inoculation on *Vigna radiata* L. Minimum amount of leghemoglobin was obtained in control and maximum amount of this protein was observed when both Fe and Mo were applied at 60 ppm and 6 ppm, respectively. In the bacteria, nitrogenase and nitrate reductase contain FeS clusters and the former has the co-factor Mo-Fe at the active site for N2 reduction.

The nitrogenase enzyme is needed to convert elemental nitrogen into a form that the plant can use. The nitrogenase enzyme complex consists of two parts. one has Mo at the center and the other has Mo and Fe at the center. The presence of molybdenum in the structure of the nitrogen-reducing enzyme indicates that legume plants need molybdenum to reduce nitrogen. Even if the plants form enough nodules, they cannot reduce nitrogen if they lack molybdenum. Nitrogen binding activity in nodules is also related to the presence or absence of leghemoglobin. Iron is found in the structure of nitrogenase enzyme, leghemoglobin and ferredoxin (Özdemir, 2002). For these reasons, legume plants need to get enough iron and molybdenum. In case of deficiency, fertilization should be done. Khan et al. (2014), in which they investigated the effect of chickpea on iron and molybdenum fertilization in Pakistan, stated that the fertilizers used increased both nodulation and yield and yield elements in a positive and significant way. Togay et. al. (2015) reported that molybdenum highly significantly and iron significantly affected the number and dry weight of nodules plant-1 in lentil as compared with control. Togay et al. (2015) investigated the effects of different doses of iron (0, 5, 10 and 20 kg ha-1) and molybdenum (0, 2,4 and 6 g kg-1 seed) on yield, yield criteria and number of nodules in lentils. They reported that the highest yield was obtained from the application of 20 kg/ha of iron and 6 g / seed molybdenum also the maximum nodules of 8.5 to 9.9 and dry weight of 7.8 to 9.1 mg plant-1 under Fe applied of 20 kg ha-1 and 7.6 to 8.8 number of nodules with a dry weight of 6.1 to 6.5 mg plant-1 under treatment of Mo at of 6 g kg-1 seed. Aktaş (2017), in his study conducted in 2017 by applying three different phosphorus (0, 4 and 8 kg/da) and three different iron doses (0, 1 and 2 kg/da) in chickpea, showed that the highest yield and nodule number were 1kg/da iron and 4 kg/da. He also stated that the other yield characteristics were also positively affected by these applications.

Copper plays a role in proteins that are required for N2 fixation in rhizobia (Weisany et. al., 2013). In copper deficiency, a large number of small nodules are formed in the plant (Özdemir, 2002). Özdemir (2002) stated that Zn, Mn, Cl and Co are necessary for plant growth but do not directly affect nodulation. Whereas cobalt is required for the synthesis of leghemoglobin and, thus, for the growth of legumes relying on symbiotically fixed nitrogen, is an essential mineral nutrient. It has been established that Rhizobium and other N2-fixing microorganisms have an absolute cobalt requirement whether or not they are growing within nodules and regardless of whether they are dependent on a nitrogen supply from N2 fixation or mineral nitrogen (Weisany et. al. 2013). Shewangizaw et.al. (2020) investigated the effects of sulfur, zinc and rhizobium applications on chickpeas. Then they reported that the highest mean nodule number (15.3) and nodule volume (1.3 ml plant-1) over locations were obtained with Rhizobium inoculation integrated with 15 kg S and 1.5 kg Zn ha-1 which resulted in 37.8% and 116.7% increment over the control check, respectively. It was also observed that combined application of Rhizobium and 30 kg S ha-1 caused the highest (6.7) mean nodulation rating and seed yield (1775.5 kg ha-1) over locations which resulted in 86.1% and 28 % increase over the control check, respectively.

CONCLUSION

The contribution of environmentally friendly leguminous plants to the sustainability of agriculture and human and animal nutrition is a well-known fact all over the world. One of the most important benefits of these plants is that they can bind the free nitrogen of the air through the rhizobium bacteria in the nodules they form in their roots. In order for them to perform these extraordinary skills more effectively, some environmental factors must be provided. At the beginning of these factors, the structure of the soil in which they are grown and the content of macro and micronutrients are very important. Different studies have been carried out for many years on the relationship between macro and micronutrients and rhizobium bacteria. Most of the studies are on nodulation efficiency, yield and yield-related properties, and there are quite a lot of studies at the molecular level. In the light of the studies carried out, by examining the legume-rhizobium-nutrient relations, in order for this relationship to become even more beneficial, we must continue to work on what to do next.

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CHAPTER 5

A REVIEW OF CO-INOCULATION STUDIES INCLUDING PHOSPHORUS SOLUBILISING BACTERİA IN CHICKPEA (*Cicer arietinum* L.)

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INTRODUCTION

Chickpea is rich in protein and provides a cheap but nutritious food with less external inputs requirment especially for low income people in many countries. Its low input and low yield production system may be further supported by biofertilizers including rhizospheric fungi and bacteria which are able to reproduce in soils for a long period of time. Their well known benefits for their hosts include improvement of tolerance to drought, salinity, insects and diseases. Soil is an environment for complex interactions between plants, soil and microorganisms like bacterias, fungi, actinomycetes, algaes and protozoa. Combining different microbial inoculants serve an additional capacity to benefit more via synergistic interactions between these partners.

In this chapter, we evaluated the international published articles on co-inoculation of chickpeas with beneficial microorganisms by putting phosphorus solubilising bacterias in the core.

Chickpea (*Cicer arietinum* L.) is a very important cool-season crop which provides nutritious food for the expanding global population and it will probably become more important with climate change (Bicer and Yilmaz, 2013; Merga and Haji, 2019). Its production is less demanding to external inputs compared to cereals. Chickpea is an important species in the cropping patterns supplying cheap protein diets especially for low income people. But low yields are declining its acceptability of this crop (Baloch and Zubair, 2010)

Biofertilizers have been used to improve availability of plant nutrients in agriculture (Han and Lee, 2006). Many rhizospheric fungi and bacteria known as plant growth promoting microorganisms have ability to infect plant roots to provide benefits like drought and salinity tolerance to their hosts (Groppa et al., 2012). Land plants engage with soil bacteria and fungi for plant survival and growth (Siebers et al., 2018). Soil acts as a medium for a wide variety of microorganisms like bacterias, fungi, actinomycetes, algaes and protozoa. Complex interactions between soil, plants and microorganisms results with different associations (Kumar, 2016). Combining different microbial inoculants is a fine method to improve nutrient availability effectively in soil (Najjar et al., 2012). As an example, from the interaction between legumes, rhizobial and arbuscular mycorrhizal partners, plants obtains nutrients and tolerance to water stress (Stancheva et al., 2017). Synergistic interactions between legume plants and bacterial endophyte rhizobias help to nitrogen nutritioning (Subramanian et al., 2015). Plant-related microorganisms serve important functions for plant health and growth. Direct promotion of plant growth by microbes bases on modified nutrient acquisition and stimulation of hormons. Other mechanisms also act in the suppression of plant pathogens with this mechanism connected indirectly with plant growth (Berg, 2009). Significanlylt higher grain yields can be obtained by interactions between plants and growth promoter microorganisms. The *Bradyrhizobium* spp. fix atmospheric nitrogen (N) in soybeans, Azospirillum induce the synthesis of phytohormones (Fipke et al., 2016), Stenotrophomonas, Bacillus, Serratia, Pseudomonas, and

Streptomyces are containing different types of beneficial bacterias and fungal genera Coniothyrium, Ampelomyces and Trichoderma influence plant growth and health (Berg, 2009).

Phosphate solubilising bacteria (PSB) strains were isolated from nodules, roots and rhizosphere of chickpea in a study of Gull et al. (2004). Their capacity of solubilisation of tricalcium-phosphate and also effect on growth of chickpea were determined. Measurements of a clear zone around the colonies indicated zones ranged from 21-83 mm. The plant growth, shoot phosphorus and nitrogen concentrations, nodulation efficiency and nitrogenase activity were significantly improved. Phosphate solubilising bacterial strains Ca18, CPS2 and CPS3 were produced maximum positive effect on shoot dry weight and nodulation of chickpea plants.

1. STUDIES CONDUCTED ON THE CHICKPEA (*Cicer arietinum* L.) ON THE SUBJECT

Zaidi and Khan (2007) inoculated Chickpeas in pots in sterilised phosphorus-deficient soil in combination with nitrogen fixing bacteria (*Mesorhizobium ciceri)* and phosphate solubilising bacteria *Serratia* (T1) or P-solubilising fungus *Penicillium* (WF6). Co-application “T1” and sole application of arbuscular mycorrhizal fungus *Glomus fasciculatum* were best. *Mesorhizobium ciceri* + *Serratia* + *G. fasciculatum* were increased the seed yield by 41% and *Mesorhizobium ciceri* + *Penicillium* + *G. fasciculatum* were increased the seed yield by 23%. P status of soil was improved by *Serratia* co-inoculated with

Mesorhizobium ciceri and *G. fasciculatum* but N contents in general were similar.

Meena et al. (2010) inoculated chickpeas with symbiotic fungus (*Piriformospora indica)* with the phosphate solubilising bacteria *Pseudomonas striata*. Single inoculation of bacteria *P. striata* and fungus *P. indica* were reduced plant growth and yields of chickpea. Combination of two microorganisms were resulted with a synergistic effect on population of bactaria *P. striata* and plant dry biomass compared to single inoculation. However, the P uptake was similar in single or combined inoculation. 20 days after sowing, in combined inoculation, population of *P. striata* was higher but 60 days after sowing, populations were similar with single and combined inoculation.

Singh and Prakash (2012) were isolated natural PSB from sandy loam soils cropped with chickpea in Punjab, India. They isolated 31 bacterial isolates showing P solubilizing activities on Pikovskaya agar medium. Isolates from genera *Pseudomonas* and *Serratia* showed positive correlation for activity of soil phosphatase and P solubilizer population and available P.

Totally 24 phosphate solubilising bacteria isolates were extracted from different Chickpea growing areas of India in a study of Dakshayini et al., (2020). Based on zone of solubilization, 10 isolates were selected under in vitro conditions. Isolate ACP-3 were higher in PSE (189%) followed by ACP-2 (150%) and CCP-2 (145%). Highest percent Pi released from tricalcium phosphate by the isolate ACP-3 (17.4%)

followed by CCP-2 (14.1%). Results showed that ACP-3 (*Bacillus licheniformis*) was the most efficient phosphate solubilising bacteria.

There is a wide gap in understanding the relationship between various soil properties and these potential bacteria or their activities. Thus, studying nitrogen fixing and phosphate solubilising bacterial populations in the rhizosphere and non rhizosphere in chickpea is important (Sumangala and Dayanand, 2014).

In the study of Sumangala and Dayanand (2014), Rhizobium population was recorded to be higher in the rhizosphere of chickpea in a black soil than azotobacter population. However, studied black soils contained more number of rhizobia and azotobacter compared to the red soils. Phosphate solubilising bacteria were higher in number in red soils compared to black soils for chickpea.

In a field experiment conducted by Gupta et al. (2009), two levels of P from two sources (Diamonnium Phosphate and Rock phosphate each at 20 kg P2O5/ha and 40 kg P2O5/ha) applied with two different species of P solubilizing bacteria (*Bacillus polymyxa* and *Bacillus megaterium*) in chickpea. Nodule dry weight, grain and straw yields and nitrogen and phosphate uptake were highest with DAP application at 40 kg P2O5/ha with co-application of *B. polymyxa* and with *B. megaterium*. Phosphate use efficiency and relative effectiveness of both P types were increased after inoculation with both phosphate solubilizing bacteria inoculants.

Singh et al. (2018) was conducted an experiment in India during Rabi season to determine the effects of PSB and rhizobium inoculation on

Chickpea. Number of nodules varied between 8-25 at 30 days and 12-43 at 60 days after sowing. Grain yields were varied between 590-1.270 kg/ha and stover yields were between 670-1700 kg/ha. Dual inoculation of Rhizobium + PSB resulted with maximum N and P uptake in grains and as well as straw.

A field experiment was conducted by Panda (2015) on a sandy loam soil with four moisture conservation techniques (control, straw mulching at 5t/ha, kaolin at 6% and Phenyl mercuric acetate at 0.3Mm). Also four levels of biofertilizer inoculation (no inoculation, P solubilizing bacteria, mycorrhizae and PSB+VAM) were applied. Result showed that pod number/plant, grain number/plant and grain weight were highest in treatments which received straw mulch and dual inoculation of PSB+VAM. Grain yield was highest (1,6 t/ha) with straw mulch. Lowest grain yield (1,2 t/ha) was obtained from control parsels.

Compatibility of VAM fungus (*Glomus fasciculatum*) with *Rhizobium leguminosarum* and PSB and their effect on plant growth attributes of chickpea were studied by Ahmed et al., (2003). Over uninoculated controls, inoculation with the local isolate increased plant height 20.17%, root length 45.34% and root colonisation up to 920%. Native strain of *Rhizobium* showed maximum efficacy compared to other tested two strains.

Co-inoculation of *Rhizobium* with phosphate solubilizing bacteria *P. striata* or *Bacillus polymyxa* werre conducted with or without chemical fertilizers on chickpea under greenhouse conditions by Alagawadi and Gaur (1988). Single inoculation with *Rhizobium* increased the nodulation and nitrogenase activity, wheras, P solubilizers increased P content of soil. Inoculation of *Rhizobium* + *P. striata* or *B. polymyxa* significantly increased the parameters of dry matter content, grain yield and N and P uptake compared to control (uninoculated).

In the study of Rudresh et al., (2005), nine *Trichoderma* spp. isolates were investigated for their capacity to solubilize insoluble P in comparison to an efficient P solubilizing bacteria (*Bacillus megaterium* subsp. *Phospaticum)*. All isolates of *Trichoderma* were solubilized insoluble tri-calcium phosphate at different levels. *Trichoderma virens* (PDBCTVs 12 and 13) and *Trichoderma viride* (TV97) solubilized 70% of the amount solubilized by reference *Bacillus megaterium*. Chickpea plants showed significantly increased P uptake when rock phosphate as the P source treated with *T. harzianum* (PDBCTH10), which was followed by *T. virens* (PDBCTVs 12) and *T. viride* (TV97).

Elkoca et al. (2007) studied the effect of chickpea seed inoculation with *Rhizobium*, nitrogen fixing *Bacillus subtilis* (OSU-142) and phosphate solubilizing *Bacillus megaterium* (M3) in Erzurum, Turkey. All inoculation combinations with *Rhizobium*, *B. subtilis* and *B. megaterium* significantly increased biomass, seed yield, seed protein content, shoot dry weight, root dry weight, nodule dry weight, plant height and pod number compared to the control treatment. Rhizobium including combined treatments were resulted with higher nodulation than sole *Rhizobium*. Seed yield increase by *Rhizobium* treatment were 18% higher but was 30.5% higher by *Rhizobium* + OSU142 + M3 compared to control.

Synergistic effects of N fixing and phosphate solubilizing rhizobacterias on chickpea plants were determined in the study of Wani et al. (2007). Grain yield and concentration and uptake of N and P were significantly increased by co-inoculation with *Mesorhizobium* and P solubilizing *Pseudomonas* and *Bacillus* spp. Inoculation with *Mesorhizobium ciceri* RC4 + *A. chroococuum* A10 + *Bacillus* PSB9 tripled the seed yield and resulted with highest grain protein content (295 mg/g) at 145 d after sowing (DAS). P uptake was highest (2.1-fold above the uninoculated control) with triple inoculation of *Mesorhizobium ciceri* RC4 + *A. chroococcum* A10 + *Bacillus* PSB 9 at 145 DAS. Study revealed that multiple inoculations with rhizospheric microorganisms can promote plant growth, increase grain yield, increase concentrations and uptake of N and P in chickpea.

The combined inoculation of P solubilizing bacteria and mycorrhizal fungus study was conducted by Saxena et al. (2015). P solubilization efficiency was highest at *Bacillus* sp. Also phosphate solubilization efficient bacteria strain RM-2 produced chitinase which may have biocontrol properties on chickpea. Dual inoculation was significantly better performed than control and single inoculations in pot trial.

Valverde et al. (2007) carried out a study in Spain to find useful biofertilizers by testing chickpea nodulating rhizobia “C2/2” and P solubilizing bacterial strain “PS-06”. 16S rDNA sequence analyses revealed that the species were *Mesorhizobium ciceri* and *Pseudomonos jessenii*, respectively. Plants inoculated with *Mesorhizobium ciceri* (C2/2) alone under greenhouse conditions produced highest for shoot dry weight. Inoculation with the race of *P. jessenii* (PS-06) produced 14% higher shoot dry weight compared to uninoculated (control) treatment without correlation with shoot P contents. Results shown that *P. jessenii* (PS-06) have ability to act in synergy with *Mesorhizobium ciceri* (C2/2) to promote growth of chickpea.

CONCLUSION

Most of the fertiliser phosphorus gets fixed by soils after applications to fields. Diversified types of benefits may be obtained by co-inoculation of symbiotic (such as *Rhizobium*) or free nitrogen fixing (such as *Cyanobacteria*) bacterias; phosporus solubilising bacterias; phosphate solubilizing fungi; drought and salt tolerance improving intracellular or arbuscular mycorrhizal fungi; mutualistic endophytic biocontrol agents (such as *Trichoderma*) against fungal diseases of plants. It is easy to see that these organisms are all good biofertilizers and growth enhancers for chickpeas when analysed worlwide articles published on that subject. Highly diversified types and strains of beneficial microorganisms are available on market and in the research infrustructures in different countries.

Outside rhizosphere, maybe in tanks or inside multiples organic/synthetic or composite membranes, mixtures of these microorganism may sustain their co-function without assitance of roots with the support of natural or synthetic root exudates. But we need to close the gap in understanding the relationship between various soil properties and these potential bacteria or their activities.

Psychrophilic (extremophilic organisms capable of growth and reproduction in low temperatures) and psychrotolerant plant growth promoting microorganisms might be co-inoculated with PSBs. Steril hydroponic conditions might be used to study co-inoculation under full seconder and micro fertilisation conditions. Co-inoculation in steril hydroponic environments may be searched more to produce special types of chickpea or other grains or byproducts of crops like alkaloids which may find place in industry or medicine, too. Not just phosphate solubilising but also P, K, Zn solubilising bacterias might be co-applied and tested in same rhizosphere in chickpea or other crop species as a group or in combination with N fixing bacterias and others.

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CHAPTER 6

THE EFFECTS OF YIELD AND YIELD COMPONENTS OF ORGANIC MANURES ON DRY BEAN (*Phaseolus vulgaris* L.) IN INOCULATION AND UNINOCULATION CONDITIONS

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INTRODUCTION

Dry bean (*Phaseolus vulgaris* L.) is one of the three food legumes, produced in the largest quantity in Turkey. Common bean or dry bean (*Phaseolus vulgaris* L.), is an important legume for human nutrition and a major protein and calorie source in the world. For dry beans, mature dry seeds are cooked, but in certain P. vulgaris varieties the immature whole pods are freshly cooked. Several common names are given to these kinds of beans in different regions including snap bean, green bean (French bean) and string bean. Agronomical practices are slightly different with different regions. Generally, for high quality yields, high plant density (25 plants/m^2) planted on top of raised beds, silty loams to moderately heavy well drained soil high in organic matter of non-salinity and near neutral pH (range of 5.5-7.5; optimum 6.5) and frequent light irrigation are suitable (Aguiar et al., 1998). Dry Bean is one of the most important protein crops in Turkey (Togay et al. 2008a).

of the grain legumes grown for food, dry bean comes foremost on the global scale with 33.066 million ha of sowing area and 289.026 million tons of produce, whereas in Turkey, it ranks third with its 88.899 ha of sowing area and 225.000 tons of produce; the mean global yield is 87.4 kg ha^{-1}, whereas this parameter is 253.1 kg ha^{-1} for Turkey (Anonymous, 2019). Dry bean is a major crop grown on fine texture alkaline soils in Turkey.

Supplementing the nutrient requirement of crops through organic fertilizers such as crop residues, manure and compost plays a key role in sustaining soil fertility and crop productivity (Soumare et al. 2003). This practice is particularly important under low input agriculture systems where nutrient availability is a serious constraint for agriculture and food production. The importance of the use of organic manures under low input agricultural systems in order to improve the quality and fertility status of soil is well documented (DeJager et al., 2001; Palm et al., 2001; Soysal et al. 2020). Manure can serve as a source of important plant nutrients including P and N (Gilley and Eghball, 2002). The land application of manure can produce crops, similar to those obtained using inorganic fertilizers (Eghball and Power, 1999). Yaduvanshi (2003) reported that the use of organic manures was widely practiced initially, but the interest declined with the increase in cropping intensity and easy availability of chemical fertilizers in the last few decades in India. Author further reported that with energy shortages, increased fertilizer cost, deterioration of soil health and environmental concerns, the use of organic manures has again become important. However, the fertilization of organic manures is reported to demand more labor power. The labor cost in developing countries is low and this technology is not uneconomical in our conditions.

Bean crop requires nitrogen in quite high amount in the first stage of development for the emergence of nodules and buildup of the symbiotic nitrogen fixation. In the later stages of development, it fulfills nitrogen requirements from bacteria. The amount of nitrogen, which symbiotically bound depends on the kind of plant, the efficiency of bacteria inoculated and soil properties (Bildirici and Yilmaz, 2005; Soysal and Erman, 2020).

The study was conducted to determine effects of different organic manures on the yield and some yield components in bean in inoculation and uninoculation conditions.

1. MATERIALS AND METHOD

The experimental field was in the city of Van located in the east region of Turkey (longitude 43° 17′E′, latitude 38° 33′N′, and altitude 1655 m). The trial was conducted in the fields of Agricultural Faculty of Yuzuncu Yıl University by using split plots design in randomized blocks with the three replications. In the study were investigated components as the plant height, first pod height, numbers of branches, numbers of pod per plant and numbers of seed per plant, numbers of seed per pod, seed yield per unit area, harvest index, biological yield, 100 seed weight, protein ratio in seed and numbers of nodule.

In 2011 precipitation throughout the season was 101.9 mm and the average over the long-term for the same period was 81.8 mm. Average temperature was 19.5^{o}C in 2011 an increase in average temperature relative to long term average of 17.6 ^{o}C. Average relative humidity was 45.4% in Table 1, (TSMS, 2011).

Table 1. Climatic data of Van in 2011 and long term.

	Precipitation (mm)		Average temperature (°C)		Relative humidity (%)	
Month	2011	Long term	2011	Long term	2011	Long term
May	62.8	45.6	13.0	13.0	59.5	56.0
June	28.1	17.7	19.2	18.1	45.9	50.0
July	11.0	5.5	23.1	22.2	39.6	45.0
Agust	0.0	13.0	23.0	17.2	36.7	44.0
Total	101.9	81.8				
Average			19.5	17.6	45.4	48.7

The soils are classified as entisols according to soil taxonomy (Soil Survey Staff, 1999). The results of calcareous soil analysis were as follows: sandy loam texture, very low in organic matter and moderate in available phosphorus (Table 2).

Table 2. Some properties of the <2 mm fraction of the top 20 cm of soil used for site

Soil properties	2011
Texture	Clay
pH[A]	8.4
Clay (%)[B]	40.2
$CaCO_3$ (%)[C]	17.9
Olsen soil test P (ppm)[D]	6.71
Total Salt (%)[E]	0.021
Organic matter (%)[F]	1.85

[A] 1 : 2.5 soil : water, [B] Bouyoucos (1951), [C] lime by calcimetric methods, [D] Olsen et al. (1954), [E] Richard (1954), [F] Jackson (1962).

The study was designed as Split Plots Design in Randomized Blocks with three replications. The plot was 12.5 m2 (2.5 m x 5 m) and included five rows and sown bean manually in rows 50 cm apart. Winter wheat was seeded at 40 seeds per square meter sown on 15 May 2011.

Kantar–05 dry bean cultivar was sown with control, DAP (14 kg/da), chicken and sheep (2 tones/da) manures.

Bean was manually harvested on 18Agust 2011. Harvest area was 6.0 m2 which excluded one row from both sides of each plot and 50 cm from both ends. Data on grain and biological yields of bean were recorded from the whole plot, but the yield components data were recorded from randomly 10 plants selected in each plot. Sub samples of the harvested grain and shoot were used to measure total Nitrogen by the Kjeldahl method (Bremner, 1965). Total N was multiplied by 6.25 to obtain % crude grain protein. The experiment was carried out under wet conditions, taking into account the rainfall, air temperature and humidity in the soil, 10 times irrigations were made.

The effect of treatments on bean were analyzed using analysis of variance procedures for a Split Plots Design in Randomized Blocks with the COSTAT statistical package. The means related with yield and yield components in bean were evaluated with Duncan’s Multiple Range Test statistical analysis.

2. RESULTS AND DISCUSSION

The analysis of variance showed that different organic manures on dry bean in inoculation and uninoculation conditions application significantly affected all the characters.

Table 3. Effect of different organic manures on dry bean in inoculation and uninoculation conditions on the yield parameters of bean*

Treatments	**Plant height (cm)**	**First pod height (cm)**	**Numbers of branches**	**Numbers of pod per plant**	**Numbers of seed per plant**	**100 seed weight (g)**	**Biological yield (kg ha^{-1})**	**Seed yield (kg ha^{-1})**	**Protein ratio in seeds (%)**	**Numbers of nodules**
Manure										
Control	31.78 c	9.71 c	5.96 c	6.13 d	25.08 d	27.43 c	3014.3b	1056.5 c	18.28c	13.68 c
Chicken	36.46 a	13.21a	8.23 a	8.23 a	34.76 a	28.73 a	3706.5a	1413.3 a	21.05a	26.98 a
Sheep	33.83 b	11.75b	7.11 b	7.66 b	31.73 b	27.93 b	3645.6a	1356.1 b	20.15 b	18.96 b
DAP	33.48 b	10.15c	6.80 b	7.18 c	29.01 c	27.76 bc	3643.6a	1321.8b	20.00 b	20.41 b
Inoculation										
Without	31.62 b	10.32b	6.60 b	6.65 b	27.13 b	27.66 b	3286.4b	1175.9 b	18.24 b	15.88 b
With	36.15 a	12.09 a	7.44 a	7.95 a	33.16 a	28.26 a	3718.6 a	1398.0 a	21.50 a	24.14 a

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

The highest plant height was obtained from chicken manure application as 34.46 cm and from rhizobium inoculation as 36.15 cm. The lowest plant height was obtained from control plots in 2011. These findings agreed with those reported by Bildirici (2003), Özçelik ve ark. (2011), Ulukan et al. (2010).

While the average first pod height obtained from the parcels without inoculation was 10.32 cm, the average first pod height obtained from the parcels where control was applied was 9.71 cm. The lowest average first pod height obtained from manures applications was obtained from the parcel treated with 10.32 cm and 0 kg / da. The highest average first pod height was obtained from 13.21 cm chicken manure application. The height of the first pod varies according to the earliness and plant height (Akçin, 1988). First pod height is a feature that is primarily affected by the genetic structure of the plant. Generally, tall plants with large vegetative parts also have high initial pod height values.

While the average numbers of branches obtained from parcels without inoculation was 6.60 number, the average numbers of branches obtained from parcels with control was 5.96 number. The average numbers of branches obtained from nitrogen dose applications varied between 5.96-8.23 numbers. The numbers of branches is a feature that directly affects the yield. Ulukan et al. (2010) reported that farm manure and bio fertilizer increase the number of branches in beans.

While the average numbers of pod per plant obtained from parcels without inoculation was 6.65, the average number of numbers of pod per plant obtained from parcels where rhizobium inoculation was applied was 7.95. The average numbers of pod per plant obtained from manures applications varied between 6.13-8.23 pieces. Numbers of pod per plant is a feature that has a direct impact on yield. Karahan and Şehirali (1999) reported that bacterial culture, inoculation and fertilizer application caused a significant increase in the number of pods in the plant compared to the control procedure.

The highest numbers of seed per plant was obtained from chicken manure application as 34.76 and from rhizobium inoculation as 33.16. The lowest seed per plant was obtained from control plots. The number of pods in the plant obtained by Karahan (1997) in the fertilization study using bacteria inoculation and different nitrogen doses and its effects on this feature are in accordance with this study.

In terms of hundred grain weight, the highest mean value was 28.26 g from bacteria-inoculated conditions and the lowest average value was 27.66 g from bacteria-free conditions. Karuç (1992) reported that the hundred grain weights obtained from the bacterial inoculated parcels were higher than the non-grafted parcels. The average values of the fertilizers ranged between 27.43-28.73 g, and the highest value was obtained from 28.73 g chicken manure. The lowest mean value was found in control with 27.43 g. Karahan and Şehirali (1999) found that bacterial culture, inoculation and fertilizer application caused significant increases in hundred grain weight compared to the control process.

The highest biological yield in bean vaccination applications was obtained from the parcels inoculated with 3718.6 kg / ha and the lowest value was found in the unvaccinated parcels with 3286.4 kg / ha. Optimum growing conditions are required for high biological yield. The number of pods and grains in the plant significantly affects the biological yield, and the plants that find a suitable growth environment develop better in the vegetative parts. The average of the fertilizers varies between 3014.3-3706.5 kg / ha. The highest biological yield was determined from chicken manure with 3706.5 kg / ha, and sheep manure and DAP manure were statistically in the same group. The lowest biological yield was obtained with 3014.3 kg/ha from the control.

The average grain yield per unit in terms of bacteria inoculation varied between 117.59-139.80 kg/da. The highest seed yield was obtained from pigeon manure application. The difference between this application and chicken manure application was found to be statistically insignificant (Togay and Ozalp 2015). Şehirali et al. (1983) reported that the grain yields obtained from the bacterial inoculated parcels were higher than the non-grafted parcels. In terms of fertilizers, the average grain yield per unit area per bean varied between 105.65 and 141.33 kg/da, and the highest unit area grain yield was determined from chicken manure with 141.33 kg/da. The lowest unit area grain yield was obtained with 105.65 kg/da from the control. Grain yield was significantly increased by applications of sewage sludge (Togay et al., 2008b). Similar results were also reported by Otieno et al. (2007), Satti et al. (2010) and Soysal (2021) reported that chicken manure increased the grain yield per unit area.

In terms of crude protein ratio, the highest average value was determined from bacteria-vaccinated conditions with 21.50%, and the lowest average value was determined from conditions without bacteria with 18.24%. In fertilizer applications, the average protein ratio in the grain varied between 18.28-21.05% and the highest value was obtained from chicken manure with 21.05%. The lowest protein content was found in the control with 18.28%. Seth et al. (2008) reported that chicken manure increases the nitrogen content in the plant. This study and our research are in harmony.

The average number of nodules obtained from the bacteria inoculation application varied between 15.88-24.14 units / plant. Bilen (2003) emphasized that bacteria vaccination increases the number of nodules. The average number of nodules in fertilizer applications varied between 13.68-26.89 pieces / plant. While the highest value was obtained from chicken manure with 26.98 pieces / plant, the lowest value of nodule number was found in the control (13.68 pieces / plant). Similar results were also reported by Otieno et al. (2007) have reported. In addition to these, Seth et al. (2008) explained that chicken manure causes an increase in the number of nodules.

CONCLUSION

While the highest seed yield per area was obtained from inoculation and chicken manure with 1539 kg/ha, the lowest value was obtained from control plots with 102h1 kg/ha. Increasing the dry bean cultivation area in Van and its surroundings depends on making bean farming attractive by increasing the grain yield per unit area. Increasing the unit area grain yield depends on the use of high-yielding, well-adapted genotypes, and the development of suitable breeding techniques for these varieties. In conclusion, considering the other characteristics discussed in the study, although 2 tons of chicken fertilization per hectare is considered appropriate for the cultivation of dry beans for the Van region, it is thought that repeating the study may be beneficial in order to achieve better practices. Information gained from this trial will be utilized to develop more efficient farm manure fertilization in bean.

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CHAPTER 7

MECHANISM OF PLANT GROWTH PROMOTION BY MICROORGANISMS AND THEIR POTENTIAL AS BIOINOCULANTS ON TEA PLANTS

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INTRODUCTION

Beneficial microorganisms improve plant nutrition and increase plant growth directly and indirectly through several important mechanisms, including fixation of elemental nitrogen, solubilization and mineralization of organic/inorganic phosphate, potassium and zinc, production of siderophores, phytohormones, ammonia, salicylic acid, hydrogen cyanide and carboxymethyl cellulose, iron sequestration, producing hydrolytic enzymes like protease, chitinase, lipase, pectinase, and cellulose, and plant tolerance to abiotic and biotic stress by lowering host ethylene levels through hydrolysis of 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC deaminase. In addition, they act by quorum sensing (QS), rhizosphere engineering, converting organic matter to biomass, reducing biofilm formation, colonizing the plant root, producing exopolysaccharides to improve aggregate, improving soil structure, and indirectly acting as a biocontrol agent by mechanisms such as inducing systemic resistance. As an alternative, the use of selected and developed effective bacterial inoculants can contribute to alleviating biotic and abiotic stress and improve yield and quality in tea by reducing production costs, and environment and other adverse impacts, as well as reducing and eliminating the use of chemical fertilizers and pesticides. This review discusses various microorganisms especially bacteria acting as PGPR, their ecology, screening strategies, working principles, applications for tea, and mechanisms and beneficial characteristics exhibited by them. This chapter is an effort to elucidate the concept of rhizobacteria and their interaction with tea plants, beneficial properties and mechanisms, with recent updates.

Tea (*Camellia sinensis* L.) is one of the world's most popular and oldest beverages, consumed by more than two-thirds of the world's population due to its appealing aroma, good taste and health-promoting effects (Kottur et al., 2011; Çakmakçı, 2016). China, the first country in the world to discover, cultivate and use tea plants of Chinese origin, is considered the home of tea and tea has become popular in more than 60 countries today and has become the most popular non-alcoholic beverage in the world (Liao et al., 2021). Leaching, harvesting shoots several times a year, the skiffing and pruning of bushes as basic cultivation practices limit the nitrogen level, which is required for tea yield and quality.

Nitrogen is widely used to increase both yields, amino acid content and quality of tea (Han et al., 2008). Several investigations have demonstrated that repeated and excessive chemical fertilization could contribute to low N utilization efficiency and cause acidification, affect nitrification rates, degradation, deterioration of soil properties, compaction and loss of fertility of tea orchard soils, potential nitrate leaching, gaseous N emissions, and changes in soil chemical composition, quality and microbial activities, as well as serious water and environmental pollution (Han et al., 2008; Hirono et al., 2009; Liu et al., 2012; Çakmakçı et al., 2017a; Tennakoon et al., 2019; Wang et al., 2019; Yang et al., 2019; Şahin and Ceritoglu, 2020). Continuous use of imbalanced inorganic fertilizers and accumulation of both root exudates and above-ground plant litters creates a highly selective environment, reduced pH, adversely affects soil microbial diversity, abundances and community composition, and the accumulation of Al toxicity and antimicrobial substances (Çakmakçı, 2019a). In fact, the decrease in soil pH causes a decline in bacterial abundance, composition, diversity and enzymatic activities (Chen et al., 2021). Due to the monoculture nature of tea plantations, tea garden ecosystems are very specific compared to other agricultural ecosystems and exert a strong rhizosphere effect on soil microflora (Pandey & Palni, 1996; Han et al., 2007; Çakmakçı, 2019a). For many years, tea cultivation and especially the overuse of N fertilizers cause problems such as soil acidification, nutrient leaching, structural damage and reduction of growth promoting microbes (Li et al., 2016; Gu et al., 2019).

Long-term tea cultivation causes monoculture problems such as soil nutrient imbalance and deprivation, accumulation of antimicrobial substances, and ototoxicity of root exudates, as well as changes in the structure and composition of the soil bacterial community, resulting in a decrease in microbial functional diversity and beneficial bacteria, promoting potentially pathogenic fungal species, and disruption in soil health processes (Zhao et al., 2012; Mortimer et al., 2015; Li et al., 2016; Arafat et al., 2019). In addition, long-term tea cultivation and soil inorganic fertilization suppress microbial respiration, reduce the functions of rhizosphere microbiota (Çakmakçı, 2019b), change the microbial community structure and biochemical and biological attributes of the soil, and reduce soil microbial diversity (Ai et al., 2015), and decrease soil pH, Ca, Mg, and K content and beneficial bacteria (Li et al., 2016; Yang et al., 2018). With the increase of continuous tea consecutive monoculture and tea planting years, soil pH, species richness, and tea yield and productivity may decrease significantly (Dutta, 2011; Arafat et al., 2019). It was determined that long-term overuse of fertilizer in tea orchards impairs nutrient absorption and use, which affects the yield and quality of tea, and some microbial enzymes are inactivated in an acidic environment (Jiang et al., 2019). In this regard, the application of chemical fertilizers reduces the soil pH and the bacterial community diversity of the tea garden soils (Ji et al., 2018; Yang et al., 2021), and the negative effects of low soil pH on bacterial diversity could be associated with leaching of nutrients and aluminium toxicity in low soil pH environment (Geisseler & Scow, 2014). Due to years of synthetic fertilizers, soil health has deteriorated, soil acidity has increased and yield and tea quality have decreased, as well as increased groundwater pollution, nitrate levels in drinking water, eutrophication, algal blooms, and 'dead zones' in coastal marine ecosystems as a result of nutrient loss caused by tea farming systems (Çakmakçı et al., 2017b). Therefore, alternative biotechnological approaches and different agricultural practices have to be adapted to increase production and maintain long-term ecological balance in agroecosystem and soil fertility.

1. MECHANISMS OF PLANT GROWTH PROMOTION

1.1. Biological nitrogen fixation

PGPR often has multiple mechanisms and improves plant growth and performance by one or more mechanisms. Biological nitrogen fixation is an important component of the nitrogen cycle and one of the most beneficial processes performed by rhizobacteria as an alternative to nitrogen fertilizer. Free-living diazotrophic bacteria can increase tea growth through phytohormone production, phosphorus release, increased nutrient intake, increased stress resistance, in addition to fixing nitrogen. In previous studies, have isolated different genera of *Bacillus, Pseudomonas, Paenibacillus, Stenotrophomonas, Arthrobacter, Serratia,* and *Burkholderia* were the most common nitrogen fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) in the tea rhizosphere, while *Bacillus pumilus, Bacillus subtilis, Bacillus licheniformis, Bacillus laevolacticus, Pseudomonas fluorescens, Stenotrophomonas maltophilia* and *Bacillus megaterium* were most commonly isolated and reported as PSB and NFB species in tea rhizosphere soils (Varmazyari & Çakmakçı, 2018). Çakmakçı et al. (2018) indicated a significant increase in growth, yield and chlorophyll, and improved N, P, K, Ca, Mg, S, Fe, Cu and Mn uptake in tea in response to co-inoculation with IAA-producing, N_2-fixing, and P-solubilizing three strains combinations (*Pantoea agglomerans* RC58 + *P. fluorescens* RC77 + *B. megaterium* RC07, and *P. fluorescens* RC512 + *B. subtilis* RC63 + *B. megaterium* RC3D). Nitrogen fixation is considered the most important feature of PGPR, which directly promotes plant growth, and free-living nitrogen-fixing bacteria can be one of the possible alternatives to mineral fertilizers, as it provides usable nitrogen to plants.

Previous research results showed that 11 of *Herbaspirillum* isolates, the predominant and most important endophytic in tea leaves, exhibited plant growth promoting (PGP) activities through N_2-fixation, P-solubilization, siderophore and IAA-production and ACC deaminase activity, while *Bradyrhizobium* isolates showed N_2-fixing, highly IAA and siderophore producing ability, but neither of them five PGPs (Yan et al., 2018). Çakmakçı et al. (2010b) tested the effect of 25 selected strains with nitrogen fixing ability on the growth and yield of tea and found that single inoculation of strains of especially *Bacillus simplex*, *B. subtilis*, *Alcaligenes faecalis*, *Staphylococcus simulans*, *Brevibacillus choshinensis*, *B. centrosporus*, *P. agglomerans* and *Paenibacillus validus* increased growth and leaf yield of Fener-3 and Hayrat tea clones under pot and field condition. Beneficial, free-living, rhizosphere and root-colonizing bacteria enhance plant growth through a wide variety of mechanisms, such as biological N fixation and solubilization and mineralization of organic/inorganic P. Some of the different uses of PGPR are listed in Table 1.

Table 1: Examples of main plant growth traits and tea crop responses by some commonly employed bacteria isolated from tea orchards

Plant growth promoting bacteria	Main plant growth-traits)	Observation/Findings	References
A. brasilense, *P. fluorescens,* *T. harzianum*	Nutrient use efficiency, enzyme activity (PO, PAL, β-1, 3-glucanase	Survival rate, plant growth, biometric parameters, key enzymes, NUE, systemic resistance	Thomas et al., 2010
B. subtilis, *B. mycoides*	Organic acid production, bacteriocinogenic property	Antifungal activity	Pandey et al., 1997
B. subtilis	Bacteriocins, enzymes, toxic substances, volatile, producing, volatile and antifungal compounds	Antifungal activity, reduced radial growth of the test fungus	Chaurasia et al., 2005
B. subtilis QM7	IAA, ammonia, siderophores production	Promote growth, root surface area and numbers of root tips	Lou et al., 2016
B. subtilis ML066-3 *B. siamensis* 122-2 *B. licheniformis*	Antibacterial activity probiotic potential.	Antagonistic activity against pathogenic bacteria	Rungsirivanich et al., 2020
B. cereus PM43, *P. aeruginosa* PM105, *Pseudomonas* sp. PM112	Growth promotion and antagonism against the target pathogen *F. lamoensis*	Shoot height, root length, fresh and dry weight, number leaves and branches, phenolic and chlorophyll content, PAL, POD, PPO activity	Morang et al., 2018
B. atrophaeus, *B. velezensis,* *B. cereus, B. flexus,* *B. thuringiensis,* *S. edaphicus,* *M. luteus*	P solubilization, IAA, ammonia, siderophore, ACC deaminase, protease production	Promote growth of rice and maize, activate antioxidative defense mechanisms, APX, SOD, chitinase, and PAL activities and accumulation of proline and polyphenols	Bhattacharyya et al., 2020
B. pseudomycoides SN29, *P. aeruginosa* KH45, *E. lignolyticus* TG1, *Burkholderia* sp. TT6	IAA, siderophore and ammonia production, P-solubilization, ACC deaminase, protease, and cellulase activity	Root and shoot biomass, root and shot length	Dutta et al., 2015
B. sediminis A6	P-solubilization, IAA, siderophore, ammonia, hydrogen cyanide, protease, chitinase, carboxy methyl cellulase production	Root elongation, length, dry and fresh weight, shoot length in rice, biocontrol activity of the isolates *R. solani*, *F. lamaensis*, *C. rolfsii*	Chopra et al., 2020
B. agri TTD5 *A. aneurinilyticus* D21 *S. koreensis* BT22 *B. megaterium* NT5	P-solubilization, IAA, ammonia, siderophore, ACC deaminase and chitinase production,	Growth of tea plant in nursery conditions	Dutta and Thakur, 2017
B. pyrrocinia	ACC deaminase, P-solubilization, siderophores and IAA-production, and grow 5% NaCl and 20% polyethylene glycol	Promote plant growth and enhance abiotic stress tolerance	Han et al., 2021
B. subtilis, *P. fluorescens*	Peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, chitinase and β-1, 3-glucanase	Root and shoot length, vigor index in rice, and reduced the disease incidence of blister bligh tea	Saravanakumar et al., 2007
Herbaspirillum sp. strain WT00C	IAA, ammonia, and siderophores production,	Stimulates tea plant growth, reduce selenate/selenite into red elemental selenium	Cheng et al., 2017

	colonizes specifically in tea plants		
P. fluorescens	Phosphate solubilization IAA, GA_3, ammonia, siderophore, HCN and salycylic acid production	Enhance plant growth, colonize plant roots and antifungal activity against tea pathogens	Ponmurugan et al., 2011
Pseudomonas spp.	Siderophore and chitinase production	Antifungal activity	Verma et al., 2007
B. cereus 932875, *P. taichungensis* 80533, *B. cepacia* 932855	N_2 fixation, phosphate and potassium solubilization	The dry shoot and fresh and dry root weights of seedlings, bud density, leaf area, hundred-bud weight, organic matter and nutrient content, water-stable aggregates, rhizobacterial community	Shang and Liu, 2021
S. marcescens	IAA, siderophore, and hydrolytic enzymes like chitinase, protease, lipase, and cellulase production	Plant growth promotion, and reduction of root-rot disease	Purkayastha et al., 2018
S. niveus, S. hyalinus, *S. camponoticapitis,* *S. sanglier,* *M. rosea,* *N. jiangxiensis,* *N. niigatensis*	P-solubilization, N_{2-} fixation, IAA, ammonia, siderophore, ACC-deaminase, protease, pectinase, cellulase, and chitinase production	Antifungal activity, enhance the growth of different tea clones in nursery conditions	Borah and Thakur, 2020
V. arenosi	Temperature, acidity/ alkalinity, desiccation, salinity, Ca, Al and Fe salts tolerance, P-solubilization, IAA, siderophore, ACC deaminaze, Gluconicand oxalic acids production,	Stress tolerance acidity, desiccation and salinity plant height, shoot dry weight, root length, and root dry weight in maize, growth parameters in tea seedlings, plant height, fresh and dry weight and yield in pea and wheat	Thakur et al., 2017

1.2. Phosphate solubilization and mineralization

Phosphorus (P) is the second most important macronutrient needed by plants, after nitrogen, which affects root development, plant growth and productivity. P plays a vital role in photosynthesis, protein formation, flower formation, energy transfer, cell division, signal transduction, biosynthesis, respiration and nutrient movement. Phosphorus is the most widely used nutrient next to nitrogen, and its main input in agriculture is by applying fertilizers. In agricultural systems, P availability and circulation of the nutrients are controlled by mineralization and immobilization, which are microbial biochemical processes in nature. In most cases, even when soil phosphorus is adequate and high and applied regularly by fertilizing, P is quickly immobilised and fixed in unavailable form (Çakmakçı et al., 2010a). Therefore, even if soils contain a high amount of total phosphorus, phosphate ions can be bound and become less soluble compounds by reacting with Al and Fe in acidic pH values, and with Ca and Mg in alkaline soils. About 70 to 90% of the applied phosphorus fertilizers are fixed and converted into inorganic form, and it is estimated that these phosphates accumulated in agricultural soils if mobilized and converted into bioavailable form through phosphate solubilizing microorganisms (PSMs), could be enough to sustain worldwide crop yields for 100 years (Walpola & Yoon, 2012; Saha et al., 2021). Most of the soil phosphorus is in the form of insoluble iron, aluminium, and calcium phosphates that cannot be utilized by plants.

Another important direct plant growth promoting property observed is the solubility of essential nutrients such as phosphorus and zinc, with microorganisms playing an important role in phosphate and/or zinc solubility thereby increasing crop yields. Microorganisms that dissolve fixed and precipitated phosphorus depending on soil pH and soil type play a vital role in the natural phosphorus cycle in the soil. Phosphorus is fixed by Ca in alkaline soils, and fixed by oxides and hydroxides, which are active forms of Al and Fe in acidic tea soils, and thus reducing its solubility (Mahdi et al., 2012). PSMs promote plant growth by converting the insoluble forms of P to soluble forms through acidification, chelation, or exchange reactions, as well as the production of organic acids. The phosphate dissolving capacity of PSB depends on the ability of these strains to produce gluconic-like acid.

Phosphorus in the soil is found in both organic and inorganic forms. Since plants cannot take P directly in organic form, it must first be converted to inorganic form available to plants after being mineralized and catalyzed by several specific enzymes such as phosphatases. This biological reaction process, which plays an important role in phosphorus cycling, is catalysed by acid phosphatases produced by both microorganisms and higher plants, and by alkaline phosphatase enzymes mainly produced by the microorganism.

Of the 36 phosphate-solubilizing strains belonging *to Bacillus, Paenibacillus, Burkholdeia, Enterobacter, Acinetobacter* and *Pseudomonas* genera, which were isolated from the rhizosphere established tea bushes using the dilution-culture method on selective media, 23 of them were capable of solubilizing inorganic phosphate and 13 of them organic phosphate (Yang et al., 2014). He found that among the predominant population in the rhizosphere soil in tea orchard, *Bacillus cereus*, *Paenibacillus* spp. and *Burkholdeia* spp. were able to solubilize both inorganic phosphate (more than 100 µg/mL) and organic phosphate (more than 40 µg/mL). According to the activity comparison results of the strains isolated from the acidic soil of tea bushes, it was found that *Azotobacter chroococcum* GD5 showed the ability to dissolve phosphate and produce auxin, while *Pseudomonas putida* GD15 showed only auxin production activity (Han et al., 2014). Inoculation with *A. chrococcum* GD5 has increased the weight of root, stem, and leaf, and nitrogen content of shoot and root of tea plant over non-inoculated control by 42%, 58%, 55%, 15% and 6%, respectively.

As a central part of the ecosystem, PSB plays a vital role in the nutrient cycle and solubilizes insoluble inorganic phosphate compounds such as dicalcium phosphate, tricalcium phosphate, hydroxyapatite, aluminium phosphate, ferric phosphate, zinc phosphate and rock phosphate through the production of organic acids. Based on the 16S rDNA sequences followed by BLAST analysis which finally revealed 70 isolates belonging to the different genera namely *Bacillus* (42), *Lysinibacillus* (5), Staphylococcus (10), *Enterobacter* (2), *Alcaligenes, Aeromonas,* and *Brevundimonas* (3) and *Escherichia* and *Klebsiella* (1) were dominant in the rhizosphere of tea (Goswami et al., 2017). Among seventy isolates, forty-seven isolates were screened for PGP properties, of which 46 were able to solubilize tricalcium phosphate, 44 to aluminium phosphate and zinc phosphate, and 42 to calcium phytate, while 34 can produce siderophores and 29 isolates HCN, 46 isolates can produce IAA and were able to solubilize both Zn and K, and 10 isolates were determined to be positive for all of these assays. On the other hand, inorganic P-solubilizing bacterium *B. subtilis* and fungus *Aspergillus niger* were found abundantly and widely in P-deficient rizosphere soil of established tea plants (Bhattacharyya & Sarmah, 2018).

Among the bacteria, *A. chroococcum, Bacillus circulans, Bradyrhizobium japonica, P. chlororaphis* and *P. putida* were isolated from the tea plantation as the most dominant P-solubilizing isolates (Bora et al., 2021). According to the isolation results based on FAME profiles and BIOLOG assays, the genera *Bacillus, Pseudomonas, Paenibacillus* and *Stenotrophomonas* were the most dominant P-solubilizing groups, while *B. cereus, P. fluorescens, S. maltophilia, B. megaterium, P. putida, B. sphaericus* and *P. polymyxa* were the most abundant P-solubilizing species in the acidic tea estates rhizosohere soils of Turkey (Çakmakçı et al., 2010a). On the other hand, PSM can be considered as an effective eco-friendly tool in establishing sustainable soil management and farming systems to increase crop production by providing soluble and available P to different plants in different agro-ecological niches.

Since almost all the phosphate-solubilizing strains produced IAA, the plant growth promoting effect of PSB may be also associated with its ability to produce plant growth regulators. In previous studies, inoculation of P-solubilizing rhizobacterial strains, namely *Bacillus firmus* strain TPB-1, *Burkholderia* sp., TPB-33, *B. firmus* TPB-38, *Burkholderia* sp. TPB-40, Burkholderia sp. TPB-57, and *B. cepacia* TPB-52, alone and in combination with hardly soluble rock phosphate in acid soils using tea plants, increased P uptake, shoot and plant biomass yield (Panda et al., 2021). The best P-solubilizing *Burkholderia* sp. and *B. firmus* performed best in both P-uptake and biomass yield in tea (Panda et al., 2021). Inoculation of P-solubilizing rhizobacterial strains *B. megaterium* increased the overall growth, the leaf number and number of branches of tea (Chakraborty et al., 2012). *Bacillus thuringiensis* B1 isolated from the red clay soil in a tea garden, able to solubilize phosphate and increase soil Olsen-available P by secreting organic acid, was inoculated onto peanuts under acidic conditions, and increased plant height, hundred-seed weight and crude protein (Wang et al., 2014a). Furthermore, Pandey et al. (2011) showed that *B. subtilis* and *P. corrugata*, and additionally arbuscular mycorrhizal fungi species of *Glomus* genus, which have antifungal and phosphate solubilizing activity, isolated from young tea rhizosphere, were also able to improve plant growth and added protection against diseases under low temperature. The beneficial PGPR applied helps to increase nutrient uptake and nitrogen fixation, to eliminate phosphorus deficiency, which is an important restriction in acidic soils due to phosphorus fixation, and to the uptake of mineral nutrients in the available form where normal plant roots fail.

1.3. Potassium solubilization

Potassium (K), along with nitrogen and phosphorus, is considered one of the three main macronutrients for tea growing. K as regulator regulates cell membrane permeability, protein synthesis, photosynthesis, stomatal opening, quality of the products and provides stress tolerance of young and mature tea plants as it forms constituents of various important enzymes of drought tolerance and water-use efficiency. In the case of K deficiency, yellow scorching or chlorosis is observed along the leaf tip margin of the dorsal surface, in severe deficiency, the leaf fired and may fall. In the researches, number of the potassium solubilizing bacteria (KSB) in the tea rhizosphere was found to be between 5.9-18.2 × 10^4 g/soil and KSB strains isolated from these soils showed high K solubility (41.91 mg/l) (Bagyalakshmi et al., 2017). On the other hand, it was emphasized that while the KSB population increased in the rainy season, it decreased in high water content of the soil and high-temperature summer periods (Panda et al., 2009). KSB or fungi dissolve insoluble silicate K minerals. It has been reported that various K-dissolving bacteria such as *Acidothiobacillus ferrooxidans*, *Paenibacillus* spp., *Bacillus mucilaginosus*, *B. edaphicus*, and *B. circulans* can solubilize K-bearing minerals such as biotite, illite, muscovite, feldspar, orthoclase, and mica (Etesami et al., 2017). Effective K-solubilizing bacteria were reported to dissolve 34-38% of total K in the insoluble form of minerals by excreting organic acids and increasing the crop yield by an average of 17% (Basak et al., 2020). Organic acids released by the activities of KSB into the root-soil interface lower the pH in the rhizosphere, which facilitates the decomposition of primary K-containing minerals. Bacteria dissolve minerals, particularly silicate minerals, and convert the insoluble and fixed K to soluble and available forms through mechanisms such as microbial production of acids, acidolysis, polysaccharides, complexolysis, chelation and exchange reactions. Organic acids released by bacteria such as tartaric, citric, succinic, α-ketogluconic, oxalic, gluconic, 2-ketogluconic, malic, propionic, and fumaric acid plays an essential role in the solubilization of K-bearing minerals.

Potassium-solubilizing microorganisms (KSM) are usually isolated from plant rhizosphere, K-rich soils and mine sites using Alexandrov medium or modified Alexandrov medium by serial dilution plate method (Basak et al., 2020). Instead of potassium aluminum silica, muscovite and biotite waste mica can also be used and after inoculation, the plates are incubated for 3-4 days at 28 ±2 °C in a biological oxygen environment. Clear colonies are selected, clear region and colony diameter are measured to find the solubilization index. Well-designed and large-scale trials should be conducted with the discovery of superior KSM strains and their efficient combinations.

About 90% of the total K is found in the soil as insoluble primary minerals, especially silicate minerals, and the solution and exchangeable K pool for crop production must be constantly recharged. Although microorganisms capable of solubilizing insoluble K in soils play an essential role in natural K cycles and catalyze K mobilization in the rhizosphere, the application of KSM is still limited (Basak et al., 2017, 2020). K affects the total chlorophyll and carotenoid content of leaves so that it could also improve crop yield through increased photosynthesis. Similarly, inoculation of the native K-solubilizing bacteria *P. putida* obtained from tea rhizosphere soils increased the yield, nutrient status of soil and shoots and dehydrogenase activity, improved all tea quality parameters from theaflavin to flavor indexes. (Bagyalakshmi et al., 2012a). Nine K-solubilizing bacterial strains were obtained from the tea garden soils, after applying the bacteria to the tea gardens, yield of tea, contents of available potassium and phosphorus in tea garden soil increased by 36.3%, 28.4% and 28.5%, respectively, the amino acid content in the tea increased, but the ratio of polyphenols/amino acids decreased, which are important in tea quality (Han et al., 2018). In another study, among the endophytic fungi isolated from tea plants, *A. niger* was found to have the most K-solubilizing and IAA-producing ability, whereas *Fusarium oxysporum* and *Penicillium sclerotiorum* had high GA_3 production and zinc solubilization, respectively (Nath et al., 2015).

1.4. Production of plant hormones

Besides nutrient-solubilizing and N_2-fixing in PGPR, an important plant growth promoting mechanism is the synthesis of phytohormones such as auxins, gibberellins, cytokinins, ethylene and abscisic acid, which are known as signal molecules in plant development (Glick, 2012; Çakmakçı et al., 2020). IAA is the most physiologically active and well-characterized phytohormone in plants, essential for plant growth and development. IAA production has been reported for various bacterial species (Hayat et al., 2010). According to the comparative review of auxin-producing bacteria (Çakmakçı et al., 2020), it has been emphasized that IAA-producing bacteria play vital role plant development, cell elongation and stimulate rooting and root formations, the lateral roots and root hairs, and the development of root and shoot as well as yield. The development of adventitious roots and the elongation or proliferation of root systems has been suggested to be one of the mechanisms by which PGPR stimulate plant growth (Çakmakçı et al., 2007; Çakmakçı & Varmazyari, 2019).

With the production of plant growth promoting phytohormones, PGPR specifically alters root formation and increases root surface area, thereby promoting nutrient uptake and plant growth. Nine strains of *P. fluorescens* selected from 250 strains isolated from the tea garden soil of various climatic regions using King's B medium have been shown to stimulate plant growth and exhibit biocontrol activity due to their PGP properties and ability to produce phytohormones (Ponmurugan et al., 2011). Furthermore, Zhan et al. (2016) showed that endophytic strain of *Herbaspirillum* sp. WT00C, isolated from tea plant, were also able to colonize in tea plants and stimulate rooting and budding due to its capability of producing IAA, ammonia and siderophores. Moreover, both IBA application and IAA-producing PGPR strains isolated from the raspberry and wheat, including *Bacillus* RC03, *Bacillus* RC23, *B. subtilis* OSU142, *P. polymyxa* RC05, *Comamonas acidovorans* RC41, *B. megaterium* RC01, and *B. simplex* RC19, can enhanced rooting percentages of tea clones, such as Pazar-20, Derepazari-7, and Tuglali-10, by producing higher levels of IAA (Erturk et al., 2008). Previous studies were recorded that inoculation of *Enterobacter lignolyticus*, which shows high PGP traits and especially high IAA (92.5 µg ml^{-1}) producer, stimulates maximum tea root and shoot growth (Dutta et al., 2015). Cheng et al. (2017) also demonstrated that inoculation with ammonia, siderophores and IAA-producing *Herbaspirillum* sp. strain WT00C bacterium leads to a stimulation in early growth without any disease symptom, and colonize especially in tea plants.

Phytohormone-producing *B. subtilis* RC11, RC63 and RC521, *B. megaterium* RC07 and RC3D, *P. fluorescens* RC77 and RC512, *P. putida* RC06, *P. polymyxa* 28/3, *P. azotofixans* 66/12, *B. cepacia* 64/4, and *P. agglomerans* RC58 have potential in field conditions to raise tea shoot development and leaf yield, nutrient uptake, and enzymes activity, and field performance and could be used for the formulation of an effective biofertilizer for tea (Çakmakçı, 2016; Çakmakçı et al., 2018). Bacterial hormones, especially IAA, play an important role in the development of the root during the early critical stages of the root. Previous studies also demonstrated the application of *P. agglomerans* PA58, *Roseomonas fauriae* RF272, *P. polymyxa* PP243, *Arthrobacter citreus* AC484, *Rhizobium radiobacter* RR112 and *B. pumilus* BP70 have resulted in significant increase in growth parameters such as plant height, number of lateral roots, root length, fresh and dry weight of root, trunk diameter, dry leaf weight, and chlorophyll content of young tea bushes (Çakmakçı & Varmazyari, 2019).

Although there are many studies on bacteria and mycorrhizal fungi as plant growth promoters, not many studies has been conducted on the plant growth promotion of yeasts. Of the 35 yeast strains, which were originally obtained from the tea plantations, 11 of them belonging to the phylum *Ascomycota* (six species, *Aureobasidium melanogenum*, *Kazachstania aquatic*, *Saturnispora diversa*, *S. sekii*, *Schwanniomyces pseudopolymorphus*, and *Wickerhamomyces anomalus*), and 24 of them belonging to the phylum *Basidiomycota* (six species, namely *Apiotrichum scarabaeorum, Curvibasidium pallidicorallinum, Papiliotrema laurentii, Rhodosporidiobolus ruineniae, Trichosporon asahii* and *T. coremiiforme*), all of them were found to produce ammonia, 28 of them were able to produce IAA, 8 of them were able to produce siderophore, 2 of them were capable of a solubilizing insoluble form of Ca and Zn (Kumla et al., 2020).

1.5. Siderophore production

Iron plays an important role in different plant biochemical processes such as metabolic pathway (fixation, TCA cycle, electron transport chain, oxidative phosphorylation, photosynthesis, respiration), biosynthetic regulation (chlorophyll, toxin, vitamins, antibiotic), and enzyme co-factor (peroxidase, superoxide dismutase, nitrogenase, hydrogenase, glutamate synthase) (Sarode et al., 2013). Iron is one of the essential nutrients for plants, and iron deficiency, in general, is due to low iron bioavailability, not low total iron concentrations in the soil. In soils, Fe is found in ferric iron (Fe^{3+}) and ferrous iron (Fe^{2+}) forms, although ferric oxide is the most abundant form in soil, plant roots absorb Fe in the form of more soluble and bioavailable ferrous iron. An antibiotic and siderophore-producing *Pseudomonas* strain isolated from iron-rich acidic tea plantation inhibited the growth of the fungus and enhanced growth in tea, pigeon pea, maize and chickpea (Kumar & Bezbaruah, 1996). In addition, siderophores play a role as signaling molecules or regulators in the interaction between plant and microorganisms, as well as iron availability and Fe nutrition of plants. Siderophores, the secondary metabolite produced by different organisms, plays an important role in the mobilization of iron and other nutrients, the solubilization of iron and its availability to plants, the biogeochemical cycle and bioavailability of iron, the receptor of iron uptake, making iron available, competition, the transport of iron, the tolerance to stress as well as a competitive strategy and promotion of plant growth.

1.6. Improvement of the soil nutrient uptake and the plant nutritional status

Seedling growth, number of leaves, their biomass and number of shoots of tea in the nursery as well as in the field were affected by inoculation with effective IAA-producing and P-solubilizing bacterial species such as *B. amyloliquefaciens*, *Serratia marcescens*, and *B. pumilus* (Chakraborty et al., 2013). Triple inoculation of N_2-fixing, P-solubilizing bacteria-based bio-fertilizers (*B. subtilis* RC521 + *B. megaterium* RC07 + *P. fluorescens* RC77 and *B. subtilis* RC11 + *B. megaterium* RC07 + *P. putida* RC06) in tea clones Fener-3 plants has been reported to enhance growth, yield as well as nutrient uptake such as N, P, K, Mg, S, Al, Fe, Cu, Mn, Zn and B (Çakmakçı et al., 2017c). These increases may be explained by mechanisms of N_2-fixation, P-solubilization, ACC deaminase, siderophore and IAA production of indigenous acid-tolerant strains isolated in the natural tea rhizosphere. Çakmakçı et al. (2015) reported that multi-traits bacterial formulations tested increased the macro (N, P, K, Ca, and Mg) and micronutrients uptake (Fe, Mn, and Zn) in tea plants under field conditions. Studies have indicated that microbial consortium improves tea growth, soil physicochemical properties, aggregation and stability, and increases nutrient bioavailability (Shang & Liu, 2021). Çakmakçı et al. (2017a) reported that inoculation of bio-fertilizer consortia encourages whole-plant growth, including plant height, trunk diameter, leaf area and yield, chlorophyll index (SPAD), and activities a wide variety of oxidative, catalytic, hydrolytic and anti-oxidative enzymes, in the Turkish registered tea clones Tuğlalı-10.

The rhizosphere microbial community structure is highly dynamic and could be affected by various factors such as plant variety, plant age, growth period, distance from soil to root, quality and quantity of root exudation pattern, soil structure and characteristics, and agricultural practices (Ali et al., 2010). Inoculated bacteria compete with indigenous bacteria, modifying native bacterial communities in the rhizosphere, which enhanced and improved the rhizosphere nutrient cycling (Mann et al., 2019). Soil microbial inhabitants associated with the rhizosphere of tea with multiple properties could be beneficial for tea production (Dutta et al., 2015). However, the interactions of these bacteria with other soil native microflora and host plants need to be explored in detail. As reported previously, inoculation with consortia of indigenous bacteria stimulated overall plant growth, including leaf yield, chlorophyll content, N, P and K concentration of tea clones Pazar-20 (Çakmakçı et al., 2018).

2. TOLERANCE AND ALLEVIATION OF STRESS IN TEA BY ASSOCIATED WITH PGPR

Factors such as temperature, heavy metal, drought, nutrient deficiency, salinity, cold, excess water, radiation, chemicals and pollutants, high light intensity, oxidative stress and wind are the main abiotic stresses that could affect tea growth and yield (Upadhyaya et al., 2013). Abiotic stresses such as temperature, heavy metal and drought are of particular importance and are the main cause of yield reduction. Providing hormonal balance, mineralization and dissolution of nutrients could promote plant-microbe interactions, as well as beneficial multi-microbial interactions, can be vital for sustainable production as it provides soil health under stress conditions as well as plant growth through biological processes (Nadeem et al., 2014). Tea production mainly depends on young succulent leaves. Chemical fertilizers are used together with pesticides and insecticides to cope with especially important leaf diseases in tea, and this application, which is dangerous for the ecosystem, is a concern in terms of pesticide residues in tea. Climatic conditions in which it is grown make tea susceptible to fungal diseases, as well as biotic factors such as drought (Liu et al., 2016), insect or pathogen (Wang et al., 2016), and heavy metals (Zhang et al., 2018) cause significant losses in tea production. Tea quality could be significantly affected by soil quality and biotic as well as abiotic stresses (Li et al., 2015). On the other hand, bacterial strains produce bioactive compounds which might positively influence soil fertility by mobilizing the fixed or unavailable P, K and Fe in the soil. Fertilization and tillage practices can change the physical and chemical properties of soils by affecting the soil bacterial community composition and diversity (Schmidt et al., 2019). As the number and efficiency of species involved in soil C, N and P nutrient cycling, which would affect tea growth, the plant and bacteria can cope with environmental disturbances through cooperation.

The raise of plant antioxidant enzymes by PGPR is an alternative approach to enhance the resistance of plants to stress (Çakmakçı et al., 2007). Previous pioneering studies, it has been shown that sugar beet, wheat, spinach and tea plants can increase glutathione reductase (GR), glutathione S-transferase (GST), peroxidase (POD), polyphenol oxidase (PPO), glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), 5-dehydroshikimate reductase (DHSK), alcohol dehydrogenases (ADH), and urease activities with PGPR inoculation (Çakmakçı et al., 2007, 2009, 2017a). If plant enzyme activity can be raised by using root-associated bacteria, it may be possible for plants to develop tolerance to stress conditions along with plant growth. With bacterial inoculation, the activity of defense enzymes such as L-phenylalanine ammonia-lyase (PAL), POD, chitinase and beta-1,3-glucanase could be increased in tea leaves (Chakraborty et al., 2013). Growth-promoting substances produced by multi-traits bacteria increase leaf ADH and PPO enzymes activity, as well as activation of other defence-related enzymes, and simultaneously increasing plant growth (Çakmakçı et al., 2015).

The tea plants inoculated with P- solubilizing, siderophore and IAA-producing *S. marcescens*, *B. amyloliquefaciens*, and *B. pumilus* showed the increased leaf defence related enzymes and induced systemic resistance, which led to the increase in height, leaf number and dry mass of leaves (Chakraborty et al., 2013). PGPR also helps to protect plants against various stresses by producing both IAA and ACC deaminase (Çakmakçı, 2009; Egamberdieva & Kucharova, 2009). In addition, PGPR could enhance plant growth and stress tolerance by reducing ethylene under stress conditions with the effect of ACC deaminase (Han et al., 2021).

2.1. Drought stress

The perennial tea plant is exposed to different environmental stresses that often affect its growth and development, one of the most important being drought. Limited water supply remains a severe environmental concern for agriculture, and drought as one of the most important environmental factors inhibits many metabolic processes and eventually restricts plant growth and crop productivity. Drought stress is abiotic stress that causes oxidative damage and changes in different physiological and biochemical processes by affecting antioxidant systems in the tea plant, which ultimately leads to significant production losses (Upadhyaya & Panda, 2004). Because of the complexity of the microbial community, little is understood regarding how climate change, such as warming, drought, and rains patterns, will affect the diversity, richness and structure of the community, although warming and drought adversely affect the physiology and bacterial biomass of the microbial community as the most common environmental stress faced by soil microorganisms (Liu et al., 2009). It has been reported that drought conditions and high temperature have a direct effect on microbial biomass, community size and composition (Frey et al., 2008; Castro et al., 2010), negatively affect microbial biomass and abundance (Kwon et al., 2013), decrease microbial abundance (Sheik et al., 2011), alter communities (Bell et al., 2009), and decrease bacterial and archaeal, but increase fungal abundance (Wang et al., 2014b). Some studies reveal that the application of K, Ca, Mn and B show some positive effect in influencing growth and antioxidative responses in the recovery period after drought, especially K and Ca show relatively better effects in improving the post-drought amelioration of the tea plant and alleviating the negative effects of stress (Upadhyaya et al., 2012).

2.2. Salinity stress

Salinity negatively affects plant synthesis and metabolism and reduces plant nutrient content. PGPR, which has the capacity to protect plants against osmotic stress, can be an important tool for alleviating salinity stress. The activity of microbes normally depends on the "environment" of the habitat, although the beneficial ones often need to compete with native microorganisms after entering into the soil, the ACC deaminase-producing PGPR, shows obvious growth promoting and salt-resistant functions on plants, increasing abiotic stress tolerance. Previous research results have shown that bio formulations of PGPR with multi-functional traits contribute to increasing yield and quality in tea under stress conditions, by improving the stress tolerance and adaptation of plants salinity stress conditions (Çakmakçı et al., 2017a). *Burkholderia pyrrocinia* strain with high ACC deaminase activity, P-solubilization and IAA-production, isolated from the tea rhizosphere, proved resistant to saline conditions and still grow weak even at 10% NaCl, stimulating the growth of peanut seedlings under salinity stress conditions (Han et al., 2021). The stress-tolerant, P-solubilizing, IAA, siderophore and ACC deaminase-producing strains *Viridibacillus arenosi*, isolated from tea rhizosphere, can enhance the stress tolerance and increase growth and yield parameters of different plants, such as maize, tea, pea, and wheat, by growing under acidity-alkalinity stress conditions, temperature, drying, salinity, and many kinds of salts (Thakur et al., 2017). Inoculation with different effective ACC deaminase, IAA- and siderophore producing, N_2-fixing, P-solubilizing, and Al-tolerant rhizobacteria isolated from tea grown soil, such as *Burkholderia anthina*, *B. cepacia* and *P. fluorescens* resulted in higher length of radicule and hypocotyl, germination of wheat and PGP activities (Ge et al., 2016). The synergistic effects of high IAA secreted by PGPR, and intracellular ACC deaminase activity are also significant for increasing the plant's tolerance to salt (Gamalero & Glick, 2015; Han et al., 2021).

2.3. Other stresses

Previous reports have shown that under nursery conditions could increase enzymes such as PAL, POD, and PPO, and plant survival, suppress brown root rot caused disease by *Fomes lamoensis*, and significantly reduce disease severity of the pathogen, while in field conditions (Morang et al., 2018). Moreover, it has been reported that the pathogenesis-related cellulase and pectinase enzymes produced by *F. lamoensis* and *Ustulina zonata* could be reduced by PGPRs (Mishra et al., 2014). Talc formulations of *S. marcescens* isolated from the tea rhizosphere, exhibiting biocontrol properties such as lytic enzymes, HCN, IAA, siderophore and antibiotic production, were efficient in controlling root rot disease caused by *Rhizoctonia solani* in tea crops and promoting plant growth (Purkayastha et al., 2018). Field trials with

PGPR such as *P. fluorescens* showed that foliar application of this organism at 7-d intervals consistently decreased the incidence of blister blight disease in tea, and increased tea yield (Saravanakumar et al., 2007). Also, in the latest study in which PSM consortia isolated from organic and inorganic tea plantation soils were tested for tolerance to pesticides, antibiotics and antifungals there was no or partial inhibition of PSM growth by some pesticides, antibiotics and antifungal agents was reported by Saha et al. (2021). Strain of *B. subtilis* obtained from the tea plantations soil has been reported to inhibit the pathogenic fungus and cause structural deformations, due to the production of diffuse and volatile antifungal compounds (Chaurasia et al., 2005). In another study found that inoculation of bacteria into tea bushes decreased the fungal population, while the increased abundance of native bacteria, actinomycetes, and nitrogen-fixing bacteria (Trivedi et al., 2005). It is interesting that bacterial isolates exhibiting the highest antifungal activity have been reported to be also the predominant bacterial strains in the tea rhizosphere (Pandey et al., 1997). Inoculation with *Bacillus, Pseudomonas, Streptomyces,* and *Trichoderma* strains isolated from tea soils increased the number of leaves and plant height as well as decreased the severity of black rot in tea plants (Islam et al., 2018). Similarly, Mishra et al. (2014) reported that single application of tea with *P. aeruginosa* and *P. fluorescens* developed induced systemic resistance against brown root rot and charcoal stump rot not only due to reduce the production of pathogenesis-related cellulase and pectinase enzymes by the pathogen, but also due to increase in the activities of PAL, POX and PPO.

CONCLUSION

It is important to research beneficial bacteria in order to develop technologies to increase inoculum efficiency, which is one of the most important sustainable practices in agriculture, and to develop new and efficient inoculants for agriculture. Microorganisms are the main components of soil and plant health that improve and enrich the nutrient quality of the soil, make plant nutrients available to the plants at low cost, increase soil fertility and crop productivity, influence plant composition, productivity and sustainability, and supplement chemical fertilizers. The microbial community components and quality indicators of tea rhizosphere soil such as abundance, biodiversity, activity and stability ensure the stability and productivity of tea production ecosystems and contribute to soil health and agricultural sustainability with its capabilities such as nutrient acquisition, fixation, mobilization, decomposition, recycling, degradation and improvement in the soil.

Plant growth promoting microorganisms can have a beneficial effect on plant growth and have been considered as one of the possible alternatives to inorganic fertilizer to promote plant growth, yield and quality. Despite all this, there is a need for extensive research on the interaction of PGPR with other microorganisms in different stress environments that are not fully elucidated, and to develop new strategies to facilitate sustainable agriculture in stressed plants and soil conditions. While it is becoming increasingly clear that beneficial microorganisms can promote plant growth by several mechanisms, extensive research is required on strategies for selecting effective strains and their ecology and rhizosphere efficiency. Currently, the effects of indigenous microorganisms on the inoculated microbial consortia are not known, and the opportunities offered and will be provided by the beneficial microbiota for the nutrition of the plant, reduction of chemical use and sustainability be not fully and adequately utilized.

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CHAPTER 8

GENETIC DIVERSITY ANALYSIS USING DNA-BASED MARKERS AND RHISOBIUM BACTERIA IN LENTILS

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INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an important cool-climate legume species that is grown primarily in Turkey, as well as in South Asia, North Africa, West Asia, North America, and Australia, mainly to be used in human nutrition (Erskine et al., 2009; Erskine et al., 2016). In general, lentils can grow better in areas with a climate above 1700-3000 meters above sea level (Agegnehu et al., 2006). The annual and self-pollinated lentil plant has a diploid (2x=2n=14) chromosome structure, and the genome size of this plant is about 4 GB (Gigabytes) (Arumuganathan & Earle, 1991). Lentil grains are rich in proteins used in human nutrition and the rest in animal nutrition, contains micronutrients, probiotics, and has an essential place in food supply and safety (Erkine et al., 1990; Toklu et al., 2009; Teng et al., 2015; Hamwieh et al., 2009). Especially the people who follow a plant-based diet prefer lentil since it is a natural source of protein, rich in natural carbohydrates, vitamins (A, B and D), and fibers. In addition, it strengthens the soil by crop rotation (alternation) (Soysal and Erman, 2020; Foti et al., 2019) and helps other field crops to fight diseases and pests. In 2017, 7.6 million tons (Mt) of lentils were obtained from an area of approximately 6.6 million hectares (Mha) in 55 countries (FAO, 2019). Lentil itself is a carbon sequester, and the Rhizobium in the roots fix the nitrogen in the free state in the air with bacteria and convert it into the form that plants can use as well. (Foti et al., 2019). Nitrogen plays a vital role in photosynthesis, as it is an element found in abundance in the chloroplasts where photosynthesis takes place. Lack of nitrogen decreases the speed of photosynthesis and restricts the plant's development (Wagner, 2012).

Soil-borne Gram-negative bacteria that fix nitrogen in the air in the nodules of Lentil roots (Asfaw et al., 2020) belongs to the *Rhizobium leguminosarum* species of the Rhizobiaceae family, *Rhizobium* genus (Garrity et al., 2005; Frank, 1989; Sprent, 2008). Ninety-two species of the Rhizobium genus live as colonies in the root nodules of lentils (Euzéby & Parte, 2021). While these bacteria living in the roots of the plant fix nitrogen in the air and convert it to ammonia, providing the plant with organic compounds such as Glutamine and ureides, compounds formed as a result of photosynthesis in the plant, benefit the bacterium reciprocally (Sawada & Kuykendall, 2003).

Capturing the nitrogen in the air by soil bacteria in the nodules of the plant roots and it is being converted into ammonia products that plants can use through various enzymatic activities is called Biological Nitrogen Fixation (Tian & Kang, 1998; Bernhard, 2010; Sprent, 2007).

Nitrogen, which is the building block of proteins, cell walls, amino acids, nucleic acids, ATP, ADP enzymes, vitamins, chlorophyll, and many more (Gardiner & Miller, 2008; McCauley et al., 2009), has the highest share (78%) in the atmosphere among all other elements.

However, not all beings can benefit from nitrogen in its form in the atmosphere. The triple bonds that form nitrogen molecules are reduced to two bonds. Nitrogen is combined with oxygen and hydrogen. Thus, the plants can use this reduced form of Nitrogen (Biological nitrogen fixation) (Uyanık et al., 2011). Sarioglu et al. (1993).

It is reported that 175 million tons/year nitrogen is fixed in plants through biological nitrogen fixation, and this is the 50% of the amount of nitrogen required by legumes.

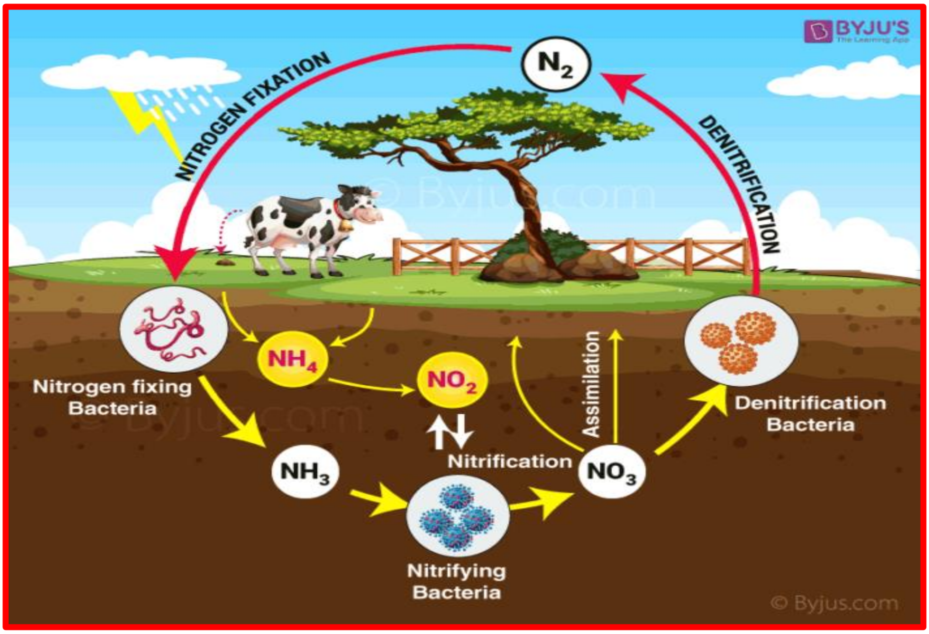

Figure 1. Nitrogen cycle (BYJU’S, 2021)

Figure 1 shows the cycle in which the Rhizobium bacteria living in the nodules in the roots of the plants fix the atmospheric nitrogen in the nodules and convert it into Nitrates (NO_{3-}) and Ammonium (NH_{4+}), the forms that plants can use. When manure, crop residues, livestock, and human tissues are mixed with the soil, the first form of nitrogen, the organic (atmospheric nitrogen) form, appears.

In this manner, Bacteria, fungi, etc., generate ammonium ions from nitrogen and convert them into ammonium (Ammonification) (Galloway et al., 2004; Smith et al., 2004). The conversion of ammonium ions in the soil to nitrite and nitrate is called "nitrification". The conversion of nitrogen into gas is called “denitrification” (Smil, 1997). The conversion of nitrogen, which is a complex compound, into a form that plants can use is called "mineralization", and the use of nitrogen taken by plants in the construction of organs and tissues, nucleic acids, and amino acids is called assimilation (Haktanır & Arcak., 2017; TEMA, 2021).

Nitrogenase is an enzyme, which plays a role in Biological Nitrogen Fixation (BAF). It is synthesized by Rhizobium and cyanobacteria and converts N_2 to ammonium (URL-1, 2021). Seefeldt et al. (2009) and Hoffman et al. (2014) reported that 8 electrons, 8 protons, and 16 ATPs are required for a molecule of N_2 to be reduced and converted into a form that the plant can use. Hardy & Havelka. (1975) acknowledged that energy has a crucial role in breaking the triple bonds of atmospheric nitrogen molecule atoms for biological nitrogen fixation, while the lack of energy has a limiting feature for fixation.

$$N_2 + 8e^- + 8\ H^+ + 16\ ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16P_i$$

Rhizobium bacteria, which fix atmospheric nitrogen (N_2), heals the soil and improve the fertility (Teng et al., 2015). The deficiency of the N_2 element (Laghari et al., 16), which is an indispensable major element for plants, causes a decrease in yield. Concurrently, using artificial nitrogen fertilizer causes eutrophication (increased nitrogen content in water), greenhouse gas effects, and acid rains (Savcı, 2012).

As a result of eutrophication, the plants irrigated with the groundwater get contaminated by the increased nitrogen affects human health negatively due to heavy metal accumulation (Addiscott, 2004). Studies have shown that the nitrogen fixed by rhizobium bacteria in legumes meets 60% of the nitrogen needed by the soil (Postgate, 1998; Postgate, 2007).

It has been observed that over 70 million tons of nitrogen are produced in this fashion (Brockwell et al., 1995), thus reducing the demand for chemical fertilizers (Peoples et al., 2009). Studies on these symbiotic bacteria in countries such as Bangladesh and Ethiopia have suggested that Rhizobium species may have different species in different regions (Lupwayi et al., 2000). Tian et al. (2010) informed that the symbiotic life between Rhizobium and lentil might be specific to lentil and rhizobium bacteria species.

Harun et al. (2009) evealed that lentil nodule bacterias (Lentil Rhizobium bacteria) are phenotypically diverse. The temperature has crucial effects on the growth and symbiotic life of bacteria and their ability to fix nitrogen (Zahran, 1999). The lentil nodule bacterium Rhizobium performs best at 33°C, while another Rhizobium variant can survive at 38°C. It is thought that this variant, which can survive at high temperatures, can be grafted onto lentil fields under high-temperature stress, thus increasing the performance of symbiotic life (Harun et al., 2009). Harun et al. (2009) revealed that the lentil nodule bacterium Rhizobium could survive in acidic and basic pH values.

1. MOLECULAR ANALYSIS

1.1. Genetic Diversity Analyses in Lentils Using Molecular Markers

Lentil is known for its low adaptation ability and low yield (Hanlan et al., 2006). Revealing the genetic diversity of lentil cultivars is critical in terms of cultivar amelioration breeding programs, conservation, and efficient use of genetic resources (Talebi et al., 2008). The use of genetic materials with minimal genetic variation for breeding purposes is the cause of stagnation in yield (Islam et al., 2012).

Over and above other desirable characteristics, parents in cultivar amelioration breeding programs in lentils should be selected from distant relatives as possible. To be able to realize this, genetic diversity analyses should be performed. The use of molecular markers in genetic diversity analyses contributes significantly to cultivar amelioration and breeding programs to provide information about the genetic diversity and relatedness of plants (Abe et al., 2003). To detect polymorphism in DNA with Molecular markers, Amplified fragment length polymorphic (AFLP) (Toklu et al., 2009; Beckmann & Soller, 1986), Random amplified polymorphic (RAPD) (Ahmad et al., 1996), Intersimple DNA-based molecular markers such as sequence repeat (ISSR) (Fikiru et al., 2007) and Simple sequence repeat (SSR) (Kushwaha et al., 2013; Weber & May, 1989) are used.

Since they are co-dominant, abundant throughout the genome, and detect medium and high polymorphism, SSRs are extensively used in genetic diversity analyses in lentils (Hamwieh et al., 2009; Jin et al., 2008; Plaschke et al., 1995). ISSR (Inter simple sequence repeat) markers are also used as molecular markers that measure differences in plants at the DNA level, thanks to their low cost, reproducibility and not requiring extensive sequence information about the genome like other SSRs (Simple Sequence repeats) (Fikiru et al., 2007; Joshi et al., 2013; Seyedimoradi & Talebi, 2014). ; Jin et al., 2008). McCarty et al. (2006) and Glaszmann et al. (2010) attribute the basis of a successful breeding program to the wide genetic variation that will enable the genotypes used as parents to be obtained from commercial, wild, and foreign genotypes. Paterson et al. (2004) and Abdurakhmonov et al. (2012) reported that narrow genetic diversity in cotton plants limits variety development breeding programs. Determining the similarities and differences between plant genotypes at the molecular level not only provides the variation needed for cultivar development breeding programs but also enables the selection of parents in segregation populations (Moose & Mumm, 2008).

1.2. Genetic Diversity Analyses

The 2g leaf sample taken from the young leaves of (14-15 days old) the lentil plant is kept at -80 or -24°C (Seyedimoradi & Talebi, 2014). DNA isolation is done using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Torres et al., 1993), and the genomic DNA is run on a 1% agarose gel. Then, the control of the genomic DNA is provided in the Ultraviolet (UV) device. SSR, ISSR, AFLP, RAPD etc. Polymerase Chain reaction with primers (PCR) is performed (Marker screening). Alleles found in PCR are scored as 1 “band avaliable”, 0 “no band avaliable”, and point (.) “missing data”. The genetic distance matrix is calculated with Popgene 1.32 (Yeh et al., 1999) computer software version. With PowerMarker 3.25 (Liu & Muse, 2005) version computer program, allele number, polymorphic allele number, polymorphism percentage, polymorphic information content (PIC; Botstein et al., 1980), genetic diversity (Nei et al., 1972), genetic distance (Nei et al., 1983) and the number of Major alleles is calculated. The phylogenetic tree is created in the MEGA_X_10.1.7 (Kumar et al., 2018) program using the data obtained from PowerMarker 3.25 and Popgene 1.32 program.

As a result of the analyses above, considering the other economic characteristics, the most distant relatives will be selected as parents to participate in the molecular breeding program to develop resistant/tolerant varieties against biotic and abiotic diseases and pests.

CONCLUSION

It is evident that soon, the world's resources will decrease so much due to the global climate change resulting from global warming, which results in, drought and irregularity of precipitation regimes and although the intensity of precipitation that causes floods and overflows remains the same, the recurrences increase, that it will be very difficult to meet the needs of the world population that uncontrollably increase.

On the one hand, reducing or zeroing the use of fossil fuels such as coal, lignite, gasoline, and diesel, on the other hand, ensuring the orientation to clean energies such as solar energy, wind energy, motion energy, and the use of new and nature-friendly alternatives to replace drugs and chemicals used in agricultural production becomes inevitable.

This study highlights the importance of taking natural nitrogen from the air by bacteria living in lentil roots, fixing, and giving it back to the plant for sustainable agriculture as the nitrogen element that is found in the structure of plants plays an active role in photosynthesis, which constitutes the essence of life.

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CHAPTER 9

BACTERIA PROMOTING PLANT GROWTH AND THEIR SECONDARY METABOLITES

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INTRODUCTION

Soil, which is an important basic resource of agriculture, contains many macro-and micronutrients necessary for plants. However, while some of these nutrients are in the form that plants can use directly, some of them are converted into forms that plants can take thanks to living microorganisms in the soil. Decreases in a quality decrease in yield and important environmental problems occur in plants that cannot receive the necessary nutrients. There are 10^{10}-10^{11} bacteria in approximately one gram of soil, and these bacteria are critical for plant health. Bacteria exert their effects by facilitating nutrient uptake, regulating plant hormone levels, and helping to counteract pathogen attacks (Claire Horner-Devine et al., 2003). For this reason, the use of beneficial root bacteria as an alternative to chemical fertilizers and pesticides in agriculture is of great importance from the point of view of sustainable agriculture. Productivity is being intensively studied (Marulanda et al., 2009; Naveed et al., 2014).

Plant-related bacteria consist of endophytic, phyllosphere, and rhizospheric bacteria (Weyens et al. 2009; Glick 2014).

1- Endophytic Bacteria: Bacterial endophytes can live in plant tissues, inside cells, or in intracellular fluids. Beneficial endophytes act indirectly by activating systemic resistance (ISR) to pathogenic bacteria (Farrar et al. 2014). It is thought that endophyte bacteria can protect the plant from abiotic stresses by improving drought stress, photosynthesis rate, and growth. (Collemare and Lebrun 2012).

2- Phyllospheric Bacteria: The phyllosphere is the outer part of the plant above the ground. Temperature, high light, and water scarcity are tolerated by symbiotic bacteria found in the phyllosphere (Weyens et al. 2009).

3- Rhizospheric Bacteria: The contact area of the soil between the root and the soil is deciphered as the "rhizosphere" (Kang et al. 2010; Tarkaka et al. 2008). It is proved that the rhizosphere is rich in bacteria. Bacteria isolated from the root zone of plants are called rhizobacteria, which stimulate plant growth. (PGPR) (Kleopper and Schroth, 1978).

PGPR bacteria directly or indirectly affect development and growth through many mechanisms. The direct effect is in the form of providing nutrients or regulating plant hormones. Indirect effect, on the other hand, includes antagonistic effects or resistance properties to pathogens. (Figueiredo et al., 2016).

Rhizosphere bacteria can be applied in different ways for biological control of plant disease and pests or for promoting plant growth. These methods include mixing the bacterial suspension into sterile soil or inoculating seeds with bacteria before planting.

PGPR can facilitate plant food intake and suppress soil-borne diseases (Bashan and de-Bashan 2010). In addition, it is known that phytohormones produced by bacteria increase tolerance to stress factors such as drought, heavy metal toxicity, extreme temperature, and salinity (Hashem et al., 2016; Sorty et al., 2016). In addition, as a result of the researches, *Acinetobacter, Brevibacillus, Pantoea, Pseudomonas, Rhizobium and Sinorhizobium, Bacillus, Enterobacter,* *Cellulosimicrobium, Mycobacterium, Ochrobactrum* and *Paenibacillus* (Egamberdieva et al., 2016)*, Azotobacter, Azoospirillum, Cellulomonas, Streptomycetes Nocardia, Nocardiopsis, Spirillospora Microcardiosporia, Micromonospora* and *Mycoplana* (Egamberdieva et al. 2009) bacteria have been reported to produce various phytohormones (Piccoli et al., 2011; de Santi Ferrara, et al., 2012; Gutierrez-Manero et al. 2001; Yandigeri et al. 2012).

1- SECONDARY METABOLITES AND THEIR IMPORTANCE

Secondary metabolites are compounds that are not indispensable for microorganisms but ensure the adaptation of the plant to the environment. In addition, it takes a partial role in reproduction and has important duties in the biotic and abiotic defense of the plant (Alvarez, 2014). The chemical structure of microbial secondary metabolites varies widely from peptides, terpenoids, polyketides, steroids, lipids, and alkaloids to carbohydrates (O'Brien and Wright 2011).

Bacteria produce a variety of secondary metabolites that have important functions, such as promoting competition, assisting in the transport of metals, or facilitating symbiotic relationships (Fang and Demain 2000). Secondary metabolite production aids plant growth, increases nutrient uptake, can alter plant sensitivity, and secures the plant. The antagonistic activity of bacteria against pathogens is due to their ability to produce different secondary metabolites, such as siderophore, HCN, IAA production, lytic enzymes, and phosphate solubility (Pandey and Maheshwari 2007; Antoun et al., 1978; Nautiyal, 1997; Biswas et al. 2000; Presmark et al., 1993; Deshwal et al. 2003;).

To observe the secondary metabolite-related gene expression change, the researchers planted *Catharanathus roseus, Curvularia sp, Choanephora infundibulifera, Aspergillus japonik, Pseudomonas sp.* were inoculated with bacterial strains. They determined that the expression of genes used for secondary metabolite production in the plant increased (Singh et al., 2020)

2- THE ROLE OF BACTERIAL SECONDARY METABOLITES IN PLANT DISEASE AND PEST CONTROL

The chemicals used in the fight against diseases and pests are expensive, as well as the negative effects on the environment and human health have led to new searches in agricultural control. To ensure agricultural productivity without harming the ecosystem, the application of PGPR in agriculture is very important for crop productivity, biological improvement, and biocontrol (Meraklı et al., 2020). Plants can acquire resistance to pathogens after exposure to stimuli by PGPR. This defense mechanism is called ISR. Induced systemic resistance (ISR) developed in response to environmental stimuli (Gouda et al., 2018). Thus, the innate defense of the plant can be strengthened against subsequent biotic challenges. The ISR defense mechanism is stimulated by various bacterial determinants, including bacterial surface components, volatile organic compounds, and bacterial secondary metabolites (DAPG, etc.) (De Vleesschauwer and Hofte, 2009).

Kempster et al. (2001) reported that *Trifolium repens* stimulated resistance against the alfalfa cyst nematode *Heterodera trifoli* when inoculated with *P. fluorescens* P29 or *B. cereus* B1. It has been reported that *P.putida* is effective against *P. deliense* and *R. solani* in tomato, in preventing disease, and in seedling development (Aşın et al., 2018). D'Errico et al. (2020) investigated the effect of applying *B. firmus* 1-1582 to *M. incognita*. In the results obtained, it was stated that *B. firmus* applications caused pressure on the nematode population. It was also found to have the lowest root gall index compared to other treatments. P. fluorescens has been reported to control up to 83.1% of G.rostochiensis and up to 86.5% of G. pallida (Nagachandrabose, 2020). It was determined that *P. fluorescens* soil application in rice parcels infested with *M. graminicola* significantly increased plant and root height, plant and root fresh, and dry weight (Subuthi et al., 2019).

Table 3.1 Biological control potential of Rhizobium spp.

Host	Producer	Target plant pathogen	References
Glycine max	*Rhizobium japonicum*	*Macrophomina phaseolina,* *Fusarium solani,*	Al-Ani et al.(2012)
Cicer arietinum	*Rhizobium sp.*	*Ciceris,* *Fusarium oxysporum sp.*	Arfaoui et al. (2005)
Arachis hypogaea	*Rhizobium Meliloti*	*Phaseolina,* *Pythium sp.,* *Macrophomina*	Bardin et al. (2004), Arora et al. (2001),

Brassica Juncea	*Mesorhizobium loti MP6*	*Fusarium oxysporum f. sp. Lentis F. solani, Sclerotinia sclerotiorum, Rhizoctonia solani, Fusarium oxysporum,*	Essalmani and Lahlou (2002), Chandra et al. (2007), Dubey and Maheshwari (2011),
Phaseolus Vulgaris	*Rhizobium sp.*	*Fusarium solani f. sp. Phaseoli*	Estevez de Jensen et al. (2002)
Arachis Hypogaea	*Rhizobium sp.*	*Sclerotium rolfsii*	Ganesan et al. (2007)
Glycine max	*Rhizobium sp.*	*Cylindrocladium Parasiticum*	Gao et al. (2012)
Lens culinaris, Pisum sativum	*Rhizobium leguminosarum bv. Viciae*	*Pythium spp.*	Huang and Erickson (2007)
Olea europaea	*Rhizobium sp.*	*Pseudomonas Savastanoi*	Kacem et al. (2009)
Cajanus cajan	*Sinorhizobium fredii KCC5*	*Fusarium udum*	Kumar et al. (2010)

Trigonella foenum-graecum	*Rhizobium Leguminosaru, Ensifer meliloti*	*Phytophthora Cinnamomi Fusarium Oxysporum,*	Malajczuk et al. (1984)
Cicer arietinum	*Rhizobium sp. NBRI9513*	*Pythium sp., Rhizoctonia bataticola, Fusarium spp.,*	Nautiyal (1997)
Glycine max	*Rhizobium sp.*	*Macrophomina Phaseolina*	Omar and Abd-Alla (1998)
Helianthus annuus	*Bradyrhizobium sp.*	*Rhizoctonia Solani*	Siddiqui et al. (2000)
Helianthus annuus	*Rhizobium sp.*	*Macrophomina Phaseolina*	Sagolshemcha et al. (2017)
Lupinus albus Vicia faba, Cicer arietinum	*Rhizobium sp.*	*Rhizoctonia Solani, Fusarium oxysporum Sclerotium rolfsii, Macrophomina haseolina, Fusarium solani,*	Shaban and El-Bramawy (2011)

Solanum lycopersicum	*Bradyrhizobium Japonicum*	*Fusarium solani, Macrophomina phaseolina, Rhizoctonia Solani,*	Siddiqui and Shaukat (2002)
Cicer arietinum	*Rhizobium Leguminosorum*	*Fusarium oxysporum f. sp. Ciceris*	Singh et al. (2010)
Cicer Arietinum	*Rhizobium sp. RS12*	*Macrophomina phaseolina*	Smitha and Singh (2014)

Singh et al. (2018)

3.1. Hcn Production:

HCN is a volatile, secondary metabolite produced by rhizobacteria (Knowles and Bunch 1986; Rezzonico et al. 2007). HCN, Siddiqui et al. (2006) as "an antagonistic factor contributing to the biological control of *Meloidogyne javanica*". Six *Rhizobium spp*. that produce HCN. it has been reported that the strain *Fusarium oxysporum* reduces the effect of chickpea wilt (Arfaoui et al. 2006). It was stated that a *P. fluorescens* CHA77 mutant, which cannot produce cyanide, did not show the nematicide activity of the wild strain (Siddiqui et al. 2006). *P. aeruginosa* PA01 has caused paralysis in nematodes by releasing hydrogen cyanide (Gallagher and Tue, 2001). HCN also shows antifungal activity against *Sclerotium rolfsii* and *Rhizoctonia solani* (Nagarajkumar et al., 2004; Rakh et al., 2011).

3.2. Siderophore Production:

Iron, ribonucleotide reductase, cytochromes, etc. It is one other the basic components of metabolic molecules (Guerinot 1994). Although it is one of the common minerals in the soil, it cannot be used directly by plants. Some microorganisms are capable of producing siderophores, a low molecular weight iron-binding compound (Andrews et al. 2003; Matzanke 1991). Bacteria that promote plant growth are one of these microorganisms. PGPR produces siderophores to retain the restricted iron present in the rhizosphere area and to obtain resistance to pathogens (Sasirekha and Srividya 2016; Sayyed et al. 2013). *M. Phaseolina* has been reported to be inhibited by strains of *Rhizobium meliloti* that produce siderophores (Arora et al., 2001). It was stated that as a result of inoculation of *Brassica campestris* seed with *Mesorhizobium loti* MP6, white rot disease was reduced (Chandra et al. 2007). According to Aydi Ben Abdallah et al. (2016) in their study, infected tomato plants with *B. tequilensis* SV104, a siderophore producing bacterium. They concluded that *Fusarium* wilt intensity decreased in grafted tomato plants and thus showed antifungal properties. Similarly, reported that it supports pepper development (Yu et al., 2011).

3.3. Lytic Enzymes: Chitinases, Proteases, and Glucanases:

One of the main mechanisms used by biocontrol agents to control pathogens is the production of enzymes that break down the cell wall structure (Kobayashi et al. 2002). Some of the lytic enzymes produced by microorganisms to reduce diseases are; cellulases, β-1,3-glucanase, amylases, β-1,4-glucanase, chitinases, β-1,6 glucanase, proteases, and pectinases. (Gupta et al. 2006; Chatterjee et al. 1995; Szekeres et al. 2004; Diby et al. 2005; Ruiz Duenas and Martinez 1996. Bacteria that synthesize these enzymes can break down the cell wall of pathogenic fungi (Budi et al. 2000). Bacteria capable of synthesizing one or more of these enzymes; *Phytophthora spp., Botrytis cinerea, Sclerotium rolfsii, Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum*. These bacteria show biocontrol activity against pathogenic fungi (Frankowski 2001). According to Sridevi et al. (2008) reported that *Rhizobium* strains isolated from Sesbania sesban produce chitinase. R. it has been reported that *leguminosarum* isolate TR2 and Ensifer *melilotie* isolate TR1 and TR4 show β-1,3-glucanase and chitinase activity. *Rhizobium sp*. RS12 strain, which can produce chitinase, has been reported to suppress the diseases caused by *F. oxysporum, S. sclerotiorum* and *M. phaseolina* in chickpea (Smitha and Singh 2014). Yoon et al. (2012) reported that chitinase and β-1,3-glucanase producing *Streptomyces cacaoi* GY525, *Meloidogyne incognita* J2 stages cause death. They also concluded that it reduced the J2 population in the soil and the number of nematode eggs in tomato roots. Similarly, Castaneda-Alvarez et al. (2016) in their study, found that B with lipase activity. *B. amyloliquefaciens* and *B. megaterium* strains have been reported to cause the death of nematodes. *Pseudomonas spp*. other lytic enzymes, such as glucanases, cellulases, and pectinases, secreted from M. it has been reported to be involved in the control of incognita (Krechel et al. 2002).

3.4. CRY Proteins:

Endotoxins that have a toxic effect on insect species and are called Cry proteins are produced by *Bacillus thuringiensis* strains (Maagd et al. 2001). As a result of various studies, it has been reported that some Cry proteins have a toxic effect on nematodes (Guo et al. 2008; Bravo et al. 2007). Cry proteins act by causing intestinal disruption in nematodes and insects (Marroquin et al. 2000). Salehi et al. (2008) reported that Cry proteins cause intestinal damage to free-living nematodes and plant-parasitic nematodes. Similarly, Höss et al. (2008) concluded that transgenic maize plants containing the CryI Ab gene significantly reduced the growth and reproduction of nematodes. It has been stated that *M. incognita* infection is controlled in tomato plants produced by the transfer of Bt Cry genes. In addition, Cry 5B toxins can affect the plant. it has been proven to strengthen the resistance of incognita to its attacks (Li et al. 2008).

3.5. 2,4-Diacetylphloroglucinol (DAPG):

Some P. it produces 2,4-diacetylfluoroglucinol (DAPG), a type of antibiotic used by fluorescent species. Bir çalışmada, DAPG üreten bir suşun domates ve soya fasulyesi bitkilerinde M. incognita hastalığını inhibe ettiği bildirilmiştir. In the same study, it was observed that a mutant strain with DAPG deficiency did not show such activity (Siddiqui and Shaukat 2003). It was found that M. incognita exposed to DAPG became more difficult to hatch (Meyer et al., 2009). DAPG, mantarlarda hücre yapısının bozulmasına neden olur (de Souza et al. 2003). Similarly, Siddiqui et al. (2003), reported that 2,4 diacetylphloroglucinol (DAPG) and hydrogen cyanide secondary metabolites produced by *Pseudomonas fluorescens* are effective on the larvae of cyst nematodes.

3.6. Volatile Organic Compounds (VOC)

Soil microorganisms are potential sources of VOCs and play a major role in the interactions between biotic and abiotic factors of the ecosystem (Bitas et al. 2013). Studies have found that Trichoderma strains produce a large number of VOCs - monoterpenes, alcohols, esters, ketones, sesquiterpenes, which positively affect plant growth and reduce the effects of the disease (Zhang et al. 2008; Hung et al. 2012; Ryu et al. 2003; Vespermann et al. 2007; Korpi et al. 2009). It has also been reported that they are plant growth promoters. In addition to their growth-promoting activities, VOCs induce systemic resistance, triggering plant tolerance to biotic and abiotic factors. The bacterial VOCs, 3-pentanol, and 2-butanone, have been reported to induce resistance against the bacterial leaf spot pathogen *Pseudomonas syringae pv* (Song et al., 2013).

It is known that more than one research has been done on the effect of antimicrobial VOCs. As a result of these studies, it has been reported that VOCs damage the DNA of pathogenic microorganisms and enzymes can change the growth of the targeted organism (Mitchell et al., 2010). It was stated that *Bacillus megaterium* YMF3.25 strain significantly reduced *M. incognita* infection by producing VOC. Similarly, *Lysinibacillus mangiferahumi* has been reported to show nematicidal activity against *M. incognita* through VOC production (Yang et al. 2012).

3.7. Lactic Acid and Amino Acids

Rhizobacterial metabolites with biological nematic for biological control include lactic acid and amino acids. Lee et al. (2014) reported that *L. capsici* YS1215 produces lactic acid (2-hydroxypropanoic acid), which inhibits the hatching of *M. incognita*. In particular, plants from the *Leguminosae* family secrete various organic compounds such as amino acids and flavonoids. It has been stated that amino acids in the culture medium of *P. macerans* cause death in the J2 stages of *Meloidogyne exigua*. It has also been observed that it reduces the nematode population in coffee plants (Oliveira et al. 2009).

3.8. Lipopeptides

Lipopeptides can be classified under four main headings: surfactins, iturines, fengycins and kurstakins (Jacques 2011). It has been reported that lipopeptides can act directly against fungi and bacteria through antibiotics, and also stimulate ISR (Ongena et al. 2007). In a study, it was stated that mutated *B. Velezensis* SQR9 strains were insufficient in the synthesis of surfactin, bacillomycin, and fengycin. By mutating *Arabidopsis* plantlets, their expression of lipopeptides was prevented. As a result of the study, it was reported that disruptions were observed in the triggering of systemic resistance induced against plant pathogens *P. syringaepv.tomato* (Pst DC3000) and *Botrytiscinerea* (Wu et al. 2018).

3.8.1. Surfactin

Surfactin has biotenside, antiviral, and antibacterial effects (Peypoux et al. 1999). Chowdhury et al. (2015) proved that surfactin also stimulates the systemic resistance pathway. It has been reported that mutant FZB42 strains lacking surfactin synthesis are impaired in the induction of ISR when exposed to the plant pathogen *R. solani* (Chowdhury et al. 2015). Magnaporthe oryzae Lolium causes a serious illness in *perenne*. It has been reported that a mutant FZB42 strain that can only produce surfactin induces systemic resistance (ISR) to *Magnaporthe oryzae* (Rahman et al., 2015)

3.8.2. Bacillomycin D

Basillomycins D is members of the iturin family. They show strong fungicidal activity. Basilomycin D showed major antifungal activity against fungal plant pathogens in *B. velezensis* strains FZB42 and C06. It suppressed spore germination and mycelium growth in *Rhizoctonia solani*, *Fusarium oxysporum* and *Monilinia fructicola* (Chowdhury et al. 2013; Koumoutsi et al. 2004). It has been reported that *bacillomycin D* shows fungicidal activity against *Fusarium graminearum*. Bacillomycin D has been reported to cause cell death in *F. graminearum.* It was observed that the pathogenicity of the plant against *F. graminearum* changed significantly when challenged with Basillomycin D. Basilomycin also triggers ISR resistance to pathogens (Wu et al. 2018).

3.8.3. Fengycin

Fengycin is known to be active against fungi. Similar to basilomycin D, its toxic effect against pathogenic fungi is based on their membrane permeability properties. The biological control exerted by the *B. velezensis* C06 strain results from the production of fengicin (Liu et al., 2011). Wu et al. (2018) reported that Fengycin triggered induced systemic resistance in *B. velezensis*.

3.9. Type I Polyketides

3.9.1. Difficidin

One of the most effective antibacterial compounds produced by *B. Velezensis* is difficidin. Difficidine is highly effective in suppressing *Erwinia amylovora,* which leads to the disease of fever blight. Similarly, the difficidin produced by Velezensis is also effective in suppressing the rice plant pathogens *Xanthomonas oryzae.* As a result of the conducted studies, it caused changes in the cell wall and structure of *Xanthomonas*, difficidin, and bacilli. In experiments on rice plants, it was concluded that difficidin and its bacillus showed the ability to suppress rice diseases such as bacterial blight and bacterial leaf spot (Borris et al., 2018).

3.9.2. Macrolactin

Macrolactin inhibits the growth of *Staphylococcus aureus* and *Enterococci* by causing disruption of cell division (Romero-Tabarez et al. 2006). Four macrolactins were identified in the culture broth of *B. Velezensis* FZB42T. These are macrolactin A and D, as well as 7-0-

malonyl and 7-0-succinyl macrolactin (Schneider et al. 2007). Macrolactin in *Arabidopsis plantlets P. syringaepv. tomato* (Pst and *Botrytis cinerea* (Wu et al. 2018).

3.10. Bacilysin

It is effective in suppressing the dipeptide Bacilysin *Erwinia amylovora*. Wu et al., (2014) stated in their study that bacilisin is very effective in suppressing *Microcystis aeruginosa*, which is the main cause of cyanobacteria growth in lakes. Similarly, they observed that it was effective in suppressing *Xanthomonas oryzae*, which causes bacterial rice blight and bacterial leaf scar. It has been reported that the suppressive effect on *Microcystis* growth is removed in a mutant strain with a disruption in the BacB bacilysin synthesis gene. However, it has been reported that bacillus is restored when its synthesis is completed (Borris et al., 2018).

4. THE ROLE OF BACTERİAL SECONDARY METABOLİTES İN THE PLANT'S DEFENSE MECHANİSM AGAİNST BİOTİC AND ABİOTİC STRESS

It is known that the presence of extreme temperatures, high light, drought, toxic metals, injuries, high salt content, environmental organic pollutants or various pathogens (viruses, bacteria, fungi, etc.) that will cause abiotic stress, adversely affect plant growth. It has been observed that PGPRs stimulate growth when the plant is in the young seedling stage and provide protection against stress factors in the periods when the plant is sensitive to environmental stresses. Thanks to PGPRs, it has been determined that the plant shows high growth and healthily overcomes stress conditions. PGPB bacteria; production of exopolysaccharides (EPS), phytohormones, 1-aminocyclopropane-1 carboxylate (ACC) deaminase, and volatile compounds, stimulate the accumulation of osmolytes and antioxidants (Guurani et al. 2013; Wang et al. 2012; Kavamura et al. 2013; Sandhya et al. 2009 Fahad et al. 2014; Glick et al. 2007; Saleem et al. 2007; Reed and Glick 2005; Vurukonda et al. 2016; Jha et al. 2011). It provides drought resistance by changing the structure of root morphology in drought tolerance (Vurukonda et al. 2016). Among PGPB, *P. fluorescens* and *Bacillus subtilis* stand out for their versatility, their capacity to grow reinforcing enzymes and training metabolites in association with root colonization and various stresses (Saravanakumar et al. 2011).

Table 4.1. PGPB phytohormonal activity in conferring drought tolerance of plants

Plant species	PGPB	Effect
Tomato	*A. brasilense*	Nitric oxide acts as a signal transmitter in the IAA
Maize	*Azospirillum lipoferum*	Gibberellins have reduced drought stress by increasing the amount of ABA.
Wheat	*Azospirillum sp.*	IAA promotes root growth lateral root growth facilitates water intake

		it facilitates nutrient uptake under drought stress
Arabidopsis	*Phyllobacterium brassicacearum*	A high ABA rate leads to a decrease in leaf transpiration
Platycladus orientalis	*Bacillus subtilis*	Cytokinin is produced ABA levels increase in shoots Increased stomatal conductivity.
Soybean	*P. putida H-2–3*	Secretion of gibberellins and plant growth increases.
Lavandula dentata	*B. thuringiensis*	By secreting IAA, it caused a higher K content. It caused a decrease in glutathione and proline.
Wheat	*Mesorhizobium ciceri, Rhizobium phaseoli and Rhizobium leguminosarum,*	The IAA has increased drought tolerance, growth and biomass rate.

Mohammadipanah and Zamanzadeh (2018)

4.1. Production of Exopolysaccharides (EPS)

EPS provides an environment that retains water and dries more slowly, thus protecting bacteria and plants from drought (Sandhya et al. 2009; Vurukonda et al. 2016). EPS also can absorb cations such as Na+. Because of these properties, it is very important for plants in saline conditions (Upadhyay et al. 2011; Shrivastava and Kumar 2015). Bacillus spp.it has been reported that the produces exopolysaccharides (EPS). Plants treated with bacteria that produce exopolysaccharides (EPS) show resistance to water and salinity stress with their ability to improve soil (Ilangumaran and Smith 2017; Ledger et al. 2016).

4.2. Metabolites with Phytohormone Effects

Phytohormones are usually synthesized in plant tissues and then transferred to their domains of action. It is effective even in very small amounts. When the hormone is delivered to the targeted tissues, it causes physiological changes such as fruit ripening, lateral root development, budding and flowering. Plant hormones are divided into five main groups: ethylene, auxins, abscisic acid, gibberellins, and cytokinins (Kang et al. 2014). Phytohormones protect plants against abiotic stress and provide resistance. PGPB can synthesize phytohormones that aid plant growth and cell division and make plants resistant to stresses (Vurukonda et al. 2016).

4.2.1. Abscisic Acid

Abscisic acid is a stress hormone that is biosynthesized in case of water shortage. ABA regulates abiotic stress and stress-responsive genes.

This ability causes resistance reactions to emerging more strongly. In addition, as a result of studies, it was stated that ABA regulates root growth and water amount in drought stress situations (Egamberdieva et al. 2017). The most important role of the ABA is to prevent sweating by stimulating the closure of the stoma (Egamberdieva et al. 2017; Vurukonda et al. 2016; Vacheron et al. 2013). ABA can trigger the development of a deeper root system to provide plants with optimal water and nutrient uptake under stressful conditions. Also, ABA maintains the hydraulic conductivity of shoot and root to use water efficiently. Thus, the retention of tissue turgor potential is provided. ABA regulates the accumulation of compatible osmolytes that maintain the antioxidant system and relative water content. It maintains the balance of ABA and other hormones including ethylene. Egamberdieva et al. (2017) reported that it provides shoot and root growth in *Zea mays*.

4.2.2. Auxins

Auxins promote plant growth by different mechanisms such as cell division, cell growth, growth rate, root formation, apical dominance phototropism, and geotropism. Olanrewaju et al. (2017), it is estimated that approximately 80% of rhizosphere microorganisms can produce and release auxin. Indole-3-acetic acid (IAA), one of the best-known auxins, is a hormone that can stimulate root elongation and lateral roots (Spaepen et al. 2007). It is thought that PGPB can support plants despite abiotic stress by providing IAA for plants (Glick 2012). Root surface area and length are increased by IAA. This feature causes an increase in water and nutrient intake. It facilitates the adaptation of plants against drought (Vurukonda et al. 2016; Shrivastava and Kumar 2015;). IAA and ACC deaminase synergistically enhance plant growth. ACC deaminase reduces ethylene-induced inhibition of plant growth. It also provides an environment where IAA can increase plant growth in both stressed and non-stressful conditions (Glick 2012). Vurukonda et al. (2016), in a study they conducted, e connected the increase in water content of wheat leaves to *Azospirillum. Azospirillum synthesizes IAA, which increases the formation of lateral roots and root growth*. Enterobacter sp., Pseudomonas sp., Pantoea sp., Marinobacterium sp., Rhizobium sp., Acinetobacter sp., and Sinorhizobium sp. produced by IAA. it has influenced the germination of wheat and seedling growth under salt stress (Meena et al. .2017).

4.2.3. Cytokinin

Cytokinins are effective in cell division in plants. Cytokinins are a class of phytohormones that promote cell divisions, cell growth, and tissue expansion. It has been stated that cytokinins can synthesize metabolites that trigger plant growth (Meena et al. 2017). Cytokinins can stimulate plant cell division and stimulate the formation of root bristles (Riefler et al. 2006). Especially during abiotic stress periods, excessive production of cytokinins significantly affects the harmful effects of abiotic stresses (Glick 2012).

4.2.4. Gibberellins

Gibberellins (GAs) regulate many physiological functions such as seed germination, stem elongation, flowering, fruiting, and senescence. They are ubiquitous plant hormones that affect different stages of plant growth (Kang et al. 2014). Kang et al. (2014) reported that GA3 and GA4 treatments restored plant growth by resisting abiotic stresses in plants. GAs increase root length and root surface area. Thus, more nutrients are absorbed. Improvement in plant function is observed under stress environments (Vacheron et al. 2013; Shrivastava and Kumar 2015). It has been reported that gibberellin is secreted by *B. licheniformis* ve *Bacillus pumilus* (Gutierrez Manero et al. 2001). Inoculation of gibberellin-producing rhizobacterium *P. putida* H-2–3 with soybean plants has been observed to induce physiological changes that lead to better growth in arid environments. Similarly, the production of ABA and gibberellin by *Azospirillum lipoferum* has been reported to reduce water scarcity stress in maize plants (Kaushal and Wani 2015). Gibberellins are associated with various plant development processes such as flowering, germination, fruit development, and stem elongation (Gomi and Matsuoka 2003).

4.2.5. Salicylic Acid,

Salicylic acid is one of the phytohormones with a phenolic structure. It plays an important role in the regulation of antioxidant enzyme activity and plant stress resistance (Egamberdieva et al. 2017). It suggests that salicylic acid is effective in inducing systemic resistance (Tahir et al. 2017). Salicylic acid governs physiological processes related to plant stress tolerance. It has been reported that the plant growth of *Sesamum indicum* plant treated with salicylic acid increases in drought conditions (Egamberdieva et al. 2017).

4.2.6. Aminocyclopropane-1-Carboxylate (ACC) Deaminase

Bacteria producing ACC deaminase reduce the amount of plant ACC. Thus, ethylene levels are also reduced. They can reduce the harmful effects of many different stresses on plants. PGPB, which produces ACC deaminase, helps to alleviate the “ethylene stress” state and stimulates normal plant growth (Mayak et al. 2004). ACC deaminase helps the activity of IAA by reducing the amount of plant ethylene.

4.3. Production of Volatile Compounds

A study by Effmert (2012) reported that 300 bacteria and fungi are VOC producers. About 800 VOCs emitted by microorganisms have been recorded. It was stated that 671 of them belong to 212 bacterial species. Bacterial VOCs play a beneficial role in three ways. These benefits are; promoting plant growth, inhibiting the growth of plant pathogens, and stimulating systemic resistance. Volatile organic compounds (VOCs) can be secreted by many PGPR strains. These secretions are low molecular weight compounds emitted from bacterial cells. (Borriss 2011, Tahir et al. 2017).

The production of volatile substances is stimulated in plants exposed to stress. Volatile compounds act as signals to initiate systemic responses in plants (Vurukonda et al. 2016). Volatile substances 3-hydroxy-2-butanone (acetoin) and 2,3-butanediol trigger plant growth, control plant pathogens, and stimulate systemic resistance (Ryu et al. 2003). Inoculation of wheat seedlings with *B. thuringiensis* AZP2 increased the survival rate of the plant by five times under intense drought.

Bacterial inoculation is thought to improve plant drought tolerance by this mechanism. Volatile substances are a good example of lightening the drought stress of the plant (Vurukonda et al. 2016). Borriss (2011) observed in a study that the mutated FZB42T strains could not support the growth of *Arabidopsis* seedlings because they could not produce volatiles. It has also been reported that VOCs have a direct inhibitory effect against pathogenic fungi (Tahir et al. 2017). *Pseudomonas chlororaphis* O6 strain inhibits water loss by producing a volatile metabolite 2R, 3R-butanediol. These volatile metabolites ensure the closure of stomata. The volatile bacterial metabolite 2R, 3R-butanediol has been reported to play an important role in promoting drought tolerance in *Arabidopsis* (Liu and Zhang 2015; Vurukonda et al. 2016).

4.4. Trehalose

Trehalose at high concentration levels is recognized as a protector against various abiotic stresses such as high temperature and salt and drought. Trehalose tolerates high temperature and acidity. Trehalose can replace water as the cells dry, forming a gel phase. This facilitates the elimination of drought and salt damage. In addition, trehalose can protect proteins from degradation that leads to high and low-temperature stresses. Treatment of plants with excessive trehalose-producing PGPB provided drought tolerance. In one study, inoculated beans with a genetically engineered trehalose production delivered more nodules, fixed more nitrogen, and formed higher biomass to the host. Maize inoculated with PGPB *Azospirillum brasilense* was modified to produce excess trehalose. As a result of the study, it was reported that plants treated with wild type *A. brasilense* were more drought-resistant and produced more biomass (Glick 2012).

5-CONCLUSİON

Pesticides, herbicides, and chemical fertilizers used in agriculture cause environmental problems. These problems have created the need for alternative plant nutrition and protection products instead of chemical fertilizers that threaten human and environmental health. Providing this need is possible with the application of organic fertilizers (biofertilizers) and the discovery and use of soil organisms. It is thought that the secondary metabolites secreted by PGPBs may be an alternative solution to the use of chemicals, since they stimulate the plant's systematic resistance to various stress factors, show antagonistic effect against pathogens in the soil, and promote plant growth and development. In this review, studies published in recent years on secondary metabolites produced by bacteria promoting plant growth and their benefits to plants are summarized. As a result, research programs and policies need to be put forward for the development of effective and safe products created from PGPB bacteria.

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CHAPTER 10

THE EFFECTS OF YIELD, QUALITY AND ENVIRONMENT OF RHIZOBIUM INOCULATION, ORGANIC AND INORGANIC MANURES SOURCES ON CHICKPEA (*Cicer arietinum* L.)

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INTRODUCTION

Chickpea is a legume plant of great value for healthy and balanced nutrition, as it is cheaper and plentiful than animal protein sources in our country. Chickpea is an edible legume plant that has been used in both human and animal nutrition since ancient times, contains protein with an average of 18-31% and 76-88% digestibility in its dry grains, rich in essential amino acids and various mineral substances (Akçin, 1988). It is known that chickpea cultivation, which has an important place in human and animal nutrition, has been carried out since 8000 years ago (Pellet, 1988). Pea's called fertile crescent where the first one culture, it is estimated that South Mesopotamia and Turkey. Thanks to *Rhizobium leguminosarum* bacteria, which live in common in chickpea roots, which is a legume plant, as a result of the air binding its free nitrogen to the soil, it leaves a nitrogen-rich soil for the plant to be planted after it. On the other hand, nutrients such as N, Ca, P, K in the roots remain in the root zone of the soil as a result of decomposition (Sepetoğlu, 2002). It is accepted that approximately 25% of the Rhizobium bacteria population naturally found in the soil is effective in biological nitrogen fixation. In order to increase this rate, seeds should be inoculated with specially prepared bacterial cultures (Pekşen and Gülümser, 1996). Soil moisture significantly affects the survival and reproduction of Rhizobium bacteria added to the soil by grafting. Too little or too much moisture in the soil causes bacteria to be largely destroyed. It is a well-known fact that increasing crop production and farmers' income depends on increasing productivity, and one of the most effective ways to improve productivity is the use of correct fertilizers. Although the share of fertilizers in productivity increase varies according to conditions, it is generally stated to be around 50% (Sepetoğlu, 1992).

Among the aims of organic agriculture, enriching biodiversity, biological cycle and biological activity in the soil comes into prominence in order to ensure the social, ecological and economic sustainability of natural systems. The effect of organic matter can be direct or indirect. Organic matter plays an indirect role as a source of plant nutrients by directly affecting the physical and chemical properties of the soil. Excessive chemical fertilization used in agriculture causes some nutrients to decrease and some to accumulate excessively. The best way to keep the organic matter in the soil at the maximum level is organic fertilization (Son et al., 2004). The use of organic fertilizers is increasing and contributing significantly to organic agriculture all over the world. Inorganic fertilizers, which are used extensively and unconsciously apart from the economic aspect, cause significant damage to the plant and the environment, especially the soil. These damages take a long time to recover and sometimes it is not possible to compensate. The fact that the chemical inputs used within the framework of increasing efficiency efforts were of a size that could threaten health caused consumer preference to change again. In this context, the organic agriculture system, which increases food reliability, has been developed within the scope of agricultural production. Considering the import of chemical fertilizers, regulating the use of inorganic fertilizers and promoting the use of organic fertilizers by considering the needs of the soils and plants are very important issues for our country and our farmers.

In this research, it was aimed to compare the effects of chemical and various organic fertilizers on the quality, yield and some yield elements of İnci chickpea variety in Fethiye conditions, with and without bacteria inoculation. The possibilities of use are aimed to be examined.

1. MATERIALS AND METHOD

In the experiment, a chickpea variety named İnci was used. This variety, which is registered by the Çukurova Agricultural Research Institute, has a plant height of 63 cm, a first pod height of 32 cm, and is suitable for machine harvesting. The grain color is beige, the grain shape is cubed. 100 seed weight of this variety is 38-42 g and average grain yield is 210-336 kg/da.

Biohumus (2% N, 1% P_2O_5, 1% K_2O, organic matter 65%), chicken manure (2.87% N, 2.35% K_2O and 2.90% P_2O_5, organic matter 56.2%) and farm manure (2% N, 1% P_2O_5, 1% K_2O, organic matter 65%), 0.85 N, 0.66% K_2O 0.14% P_2O_5, organic matter 46.2%) and worm manure (2.4% N, 1.4% P_2O_5, 1% K_2O, organic matter 49.6%) fertilizers as an inorganic fertilizer source, DAP 18% nitrogen (N) and% 46 phosphorus pentaoxide (P_2O_5) fertilizers were given.

This study was conducted in the 2018 growing season in Kayaköy, Fethiye, Muğla province. The field where the research was conducted is located in Fethiye District Kayaköy neighborhood and 2 km away from the main road. The trial area is 146 m above sea level and is located at 36° 34′ 44.8356 "north latitude, 29° 5′ 17.4228" east longitude.

In Fethiye District of Muğla Province, the winter season is rainy and mild, and the summers are dry. The Mediterranean mild climate prevails in the Fethiye district of Muğla province. Due to the location of the district on the Mediterranean coast, it is temperate with the effect of sea. The climate data for the months covering the period in which the experiment was carried out and the average of long years are given in Table 3.1. The average annual rainfall in the growing season of the region where the study was conducted is 170.3 mm, the average temperature is 20.64 ° C, and the average relative humidity is 63.329%. The amount of rainfall in 2018 is 118.64 mm. (TSMS, 2018).

Table 1. Climatic data of Mardin in 2012-2013 and long term.

	Precipitation (mm)		Average temperature (C^O)		Relative humidity (%)	
Months	2012-2013	Long term	2012-2013	Long term	2012-2013	Long term
March	67.2	84.9	15.5	13.2	70.3	67.3
April	6.2	43.3	19.5	16.4	62.2	67.1
May	28.2	28.3	23.9	20.6	59.9	65.4
June	17.0	5.3	26.4	25.1	61.7	59.4
July	----	8.5	30.1	27.9	51.4	57.4
Total	981.6	660.8				
Average			13.2	11.9	51.6	50.2

According to the results of the soil analysis of the experiment, the soil samples obtained from a depth of 0-20 cm in the research area were found to have clayey loam, slightly alkaline reaction, medium organic matter content, slightly calcareous in terms of lime content, salt-free, medium phosphorus content, and sufficient potassium content (Table 2).

Table 2. Some properties of the <2 mm fraction of the top 20 cm of soil used for site

Soil properties	2018
Texture	Clay- Loam
pH[A]	7.96
Clay (%)[B]	48.76
$CaCO_3$ (%)[C]	2.53
Olsen soil test P (ppm)[D]	12.25
Total Salt (%)[E]	0.404
Organic matter (%)[F]	2.16

[A] 1 : 2.5 soil : water, [B] Bouyoucos (1951), [C] lime by calcimetric methods, [D] Olsen et al. (1954), [E] Richard (1954), [F] Jackson (1962).

The trial was set up in 2018 with 3 replications according to the factorial trial pattern in random blocks. There are a total of 36 parcels in the trial and each parcel consists of 5 rows. The distance between rows in parcels is 30 cm, parcel area; It was planned to be 5 m x 1.5 m = 7.5 m^2. In the experiment, a distance of 2 m was left between the blocks and parcels. The amount of seed to be thrown into the parcel, 60 seeds per m^2, has been determined. Biohumus (2% N, 1% P_2O_5, 1% K_2O), chicken manure (2.87% N, 2.35% K_2O and 2.90% P_2O_5) and Farm manure (% 0.85 N, 0.66% K_2O 0.14% P) and worm manure (2.4% N, 1.4% P_2O_5, 1% K_2O) and DAP (18% N and 46% P_2O_5) fertilizer were given as inorganic fertilizer sources. DAP 14 kg / da, chicken manure 2 tons / da, farm manure 2 tons / da, worm manure 300 kg / da, bio humus 150 kg / da and control were planted in pearl chickpea variety. In the experiment, all seeds to be inoculated with bacteria were inoculated with *Rhizobium ciceri* bacteria culture, which was found to effectively form nodules in chickpeas (Öğütçü, 1998). Bacteria culture was obtained from Ankara Soil Fertilizer and Water Resources Central Research Institute. In the measurements, the plants in the 5 rows forming the parcel, one row on each side and 50 cm from the row heads, were excluded from observation as the edge effect and all operations were carried out on the areas of 0.9 m x 4 m = 3.6 m². The experimental area was plowed deeply in the autumn of 2017, a second outcrop before sowing, and then a duplicate by pulling the disc harrow and the seed bed was made ready for sowing. The sown activity was done by hand on 15.03.2018 by opening lines with a marker. Weed control in the experimental area was done with hoes twice, before and after flowering. The harvest of the trial was carried out on 08.07.2018.

2. RESULTS AND DISCUSSION

The values obtained in the study were subjected to variance analysis and the averages of the analyzed parameters were grouped according to Duncan Multiple Comparison (5%) Test.

Table 3. Plant height average of bacterial inoculation, organic and inorganic fertilization applications and Duncan groups formed (cm) *

	Manures						
	Control	Chicken	Farm	Worm	DAP	Biohumus	Mean
Uninoculation	39.1 f	49.7 c	46.2 d	46.1d	45.7 d	42.6 e	44.8 B
Inoculation	41.9 e	55.2 a	52.4 b	49.6 c	49.9 c	44.6 d	49.0 A
Mean	40.5 E	52.4 A	49.3B	48.0 B	47.8 C	43.5 D	

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

In Table 3, plant height average of fertilization in chickpeas varied between 40.5-52.4 cm. The highest plant height was obtained from chicken manure application with 52.4 cm and the lowest plant height was obtained from 40.5 cm and 0 kg/da (control) application. When the plant height of chickpeas was examined in terms of Rhizobium bacteria inoculation, the highest plant height was obtained from the inoculation application with 49.0 cm, and the lowest plant height was obtained from the un inoculation application (44.8 cm). Amin and Moghadasi (2015), in their study with different nitrogen doses (0,90,120 kg ha^{-1}) and vermicompost (0, 15 tons' ha^{-1}), were among the control plots of the lowest plant height, and the highest plant height was worm. They reported that they obtained from the applications where the fertilizer was given.

Table 4. Number of branches of bacterial inoculation, organic and inorganic fertilization applications in chickpeas and Duncan groups formed (Numbers / plant) *

	Manures						
	Control	Chicken	Farm	Worm	DAP	Bio humus	Mean
Uninoculation	2.8 f	4.6 b	4.2 c	3.8 d	3.8 d	3.2 e	3.76 B
Inoculation	3.2 e	5.7 a	5.6 a	4.9 b	4.2 c	4.0 cd	4.62 A
Mean	3.00 F	5.18 A	4.90 B	4.38 C	4.03 D	3.66 E	

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

It has been determined that the average number of branches in chickpeas fertilization varied between 3.00 and 5.18. The highest number of branches was obtained from chicken manure application with 5.18 number, while the lowest number of branches was obtained from 0 kg/da (control) application with 3.00 number (Table 4). When the number of branches in chickpeas was examined in terms of Rhizobium bacteria inoculation, the highest number of branches was obtained from the inoculation application with 4.62 and the lowest branch number was obtained from the inoculation application (3.76). Çiftçi et al. (2004) reported that the number of branches in the plant varied between 2.2-4.1. Zeidan (2007) stated that organic fertilizer application increased the number of branches and Saket et al. (2014) stated that the applied organic fertilizers (Farm, chicken, compost and vermicompost) increased the number of branches, but the difference between them was not significant. Fertilization x inoculation interaction was found to be statistically significant in terms of branch number. The highest value was determined as 5.7 bacterial inoculation and chicken manure application. There is no statistically significant difference between chicken manure application and farm manure application. The lowest value in terms of the number of branches was found to be 2.8 in the control parcel.

Table 5. Number of pods per plant and Duncan groups (pieces / plant) of chickpea bacterial inoculation, organic and inorganic fertilization applications *

	Manures						
	Control	Chicken	Farm	Worm	DAP	Biohumus	Mean
Uninoculation	17.06	23.40	221.86	28.66	19.93	18.63	20.26 B
Inoculation	21.00	26.23	25.03	24.13	23.96	22.60	23.82 A
Mean	19.03 E	24.81 A	23.45 B	22.40 C	21.95 C	23.45 B	

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

The average number of pods per plant of fertilizers varied between 19.03 - 24.81 pods / pod and the highest value was obtained from chicken manure with 24.81 pods/pod. When the effect of different vaccination applications on the number of pods per plant is examined in Table 5, it has been determined that the average values vary between 20.26 - 23.82 numbers / plant. In studies conducted on plants related to microbial, organic and inorganic fertilizer applications; Kaya et al. (2007), in their study on the effect of organic and commercial fertilizer on chickpea plant, the lowest number of pods in the plant was obtained from control plots (11.7 units), followed by commercial fertilizer application (15.2) and organic reported that the highest (17.1 numbers) values were obtained from fertilizer application.

Table 6. Number of grain per plant of chickpea bacterial inoculation, organic and inorganic fertilization applications and Duncan groups formed Numbers/ plant) *

	Manures						
	Control	Chicken	Farm	Worm	DAP	Biohumus	Mean
Uninoculation	17.6	25.0	23.2	21.4	20.7	19.4	21.2 b
Inoculation	22.1	28.3	27.1	25.8	25.3	23.7	25.4 a
Mean	19.9 e	26.7 a	25.2 b	23.6 c	23.0 c	21.5 d	

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

The average of fertilizers varied between 19.9 - 26.7 numbers/plant. While the highest grain number value in the plant was obtained from chicken manure with 26.7 pieces/plant, the lowest value was determined with 19.9 numbers/plant in the control. In terms of bacterial inoculation applications, the highest number of grain in the plant was obtained from the inoculation application with 25.4 numbers/plant, while the lowest number of grain in the plant was obtained from the bacteria-free parcels with 21.2 numbers/plant (Table 6). It is natural to obtain more seeds from plants with a high number of pods per plant. In studies on the number of grains per plant; Genetic structure of the variety, environmental conditions and applied growing techniques are effective in obtaining high yield per unit area by completing the development of chickpea plant. There is a positive and reliable relationship between the number of seeds and pods per plant and plant yield. Increasing the number of seeds and pods per plant also increases the grain yield in the plant (Güler et al., 2001).

Table 7. Grain yield per unit area average of bacterial inoculation, organic and inorganic fertilization applications and Duncan groups formed (cm) *

	Manures						
	Control	Chicken	Farm	Worm	DAP	Biohumus	Mean
Uninoculation	142.2 h	180.7bc	170.7de	164.5ef	164.6ef	154.7 g	162.9 B
Inoculation	149.6 g	200.6 a	189.5 b	174.7cd	174.1cd	161.7 f	174.9 A
Mean	145.9 E	190.4 A	180.2 B	169.6 C	169.3 C	158.2 D	

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

In terms of fertilizers, the average grain yield per unit area per chickpea varied between 145.9-190.4 kg/da and the highest unit area grain yield was determined from chicken manure with 190.4 kg/da. The lowest unit area grain yield was obtained from the control with 145.9 kg/da. As seen in Table 4.14, the average grain yield per unit area in terms of grafting varied between 162.9-174.9 kg/da. Amin and Moghadasi (2015) reported that they obtained the lowest grain yield from control plots in chickpea plants where worm fertilizer and nitrogen fertilizer applications were applied, they obtained high grain yield from nitrogen fertilizer and worm fertilizer applications, and the difference was insignificant. The highest seed yield was obtained from pigeon manure application. The difference between this application and chicken manure application was found to be statistically insignificant (Togay and Ozalp, 2015). Fertilization x inoculation interaction was found to be statistically significant in terms of grain yield per unit area. The highest value was determined as 200.6 kg/da from bacteria inoculation and chicken manure application. In terms of grain yield per unit area, the lowest value was found as 142.2 kg/ha in the control parcel.

Table 8. Harvest index of bacteria inoculation, organic and inorganic fertilization applications and Duncan groups formed (%)

	Manures						
	Control	Chicken	Farm	Worm	DAP	Biohumus	Mean
Uninoculation	23.9 h	27.0 cde	26.7de	26.0 ef	26.0ef	24.7 g	25.7 B
Inoculation	25.2 fg	31.1a	29.0 b	27.7 cd	27.1 c	26.3 ef	27.7 A
Mean	24.5 E	29.1 A	27.8 B	26.8 B	26.6 C	25.5 D	

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

In terms of fertilizers, the average harvest index of chickpeas varied between 29.1 - 24.5%, and the highest harvest index was determined from chicken manure with 29.1%. The lowest harvest index was obtained from the control with 24.5%. As seen in Table 8, the average harvest index in terms of inoculation varied between 25.7-27.7%. Togay et al. (2005) reported that the harvest index values of chickpeas varied between 35.5-41.6%. Yeşilbaş (2015) reported that he obtained the highest harvest index value from chicken manure application with 37.4% and the lowest average value from control plots with 32.8%, followed by sheep manure and DAP manure, respectively. Rudresh et al. (2005), the results obtained by researchers from their studies are similar to the findings we obtained.

Table 9. 100-grain weight averages of bacterial inoculation, organic and inorganic fertilization applications and Duncan groups formed (g) *

	Manures						
	Control	Chicken	Farm	Worm	DAP	Biohumus	Mean
Uninoculation	31.6 h	34.9 cde	34.6def	33.8efg	33.2fg	32.8 gh	33.5 B
Inoculation	34.7def	39.5 a	37.8 b	36.3 c	36.0 cd	34.2 efg	36.6 A
Mean	33.1 C	37.2 A	36.9 A	35.1 B	34.6 B	33.5 C	

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

In Table 9, while the highest 100 grain weight was obtained from chicken manure application (37.2 g), it was in the same group with farm manure application. The lowest 100 grain weight was obtained from the control application. In terms of bacteria inoculation, the highest 100 grain weight was determined with 36.6 g without inoculation, and the lowest value with 33.5 g in without inoculation application. Amin and Moghadasi (2015) reported that while the lowest hundred grain weights were obtained from the control plots in the chickpea plant where worm fertilizer and nitrogen fertilizer applications were applied, the parcels where commercial fertilizer and worm fertilizer were applied were in the same group. Soysal and Erman (2020) obtained the highest yield of 100 grains of chickpea plant from *rhizobium ciceri* + 2 kg N/da, 5 kg P2O5/da (33.8 g) application according to the average of years in their study in Siirt province conditions. They reported that they obtained the lowest efficiency from the control application.

Fertilization x inoculation interaction was found to be statistically significant in terms of hundred grain weight. The highest value was found as 39.5 g from bacterial inoculation and chicken manure application. In terms of hundred grain weight, the lowest value was found as 31.6 g in the control plot.

Table 10. Average protein ratio in the grain of chickpea bacterial inoculation, organic and inorganic fertilization applications and Duncan groups formed (%) *

	Manures						
	Control	Chicken	Farm	Worm	DAP	Biohumus	Mean
Uninoculation	19.1	23.6	22.5	22.3	21.8	19.7	21.5 b
Inoculation	20.4	24.0	23.0	22.8	22.6	20.3	22.2 a
Mean	19.7 c	23.8 a	22.7 b	22.5 b	22.2 b	20.0 c	

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

According to Duncan Multiple Comparison results, it was determined that different groups were formed between fertilizers in terms of protein content in the grain. The average protein ratio in the grain obtained from different fertilization varied between 19.7 - 23.8%. The highest protein rate in the grain was found with 23.8% from chicken manure application. The lowest protein ratio in the grain was obtained from the control with 19.7% (Table 10.). In Rhizobium bacteria vaccination, the highest protein ratio was found in the vaccination application with 22.2% and the lowest protein ratio in the non-vaccination application. Mohammed et al (2010) reported that when farm manure and compost are given in addition to chemical fertilizers in chickpeas in Iran, the protein ratio increases.

Table 11. Average number of nodules of bacterial inoculation, organic and inorganic fertilization applications in chickpeas and Duncan groups (numbers / plant) *

	Manures						
	Control	Chicken	Farm	Worm	DAP	Biohumus	Mean
Uninoculation	7.4 h	22.3 c	22.0 c	17.8 e	14.8 ff	15.0 f	16.5 B
Inoculation	10.5 g	28.4 a	28.0 a	25.3 b	18.9 d	17.3 e	21.4 A
Mean	8.9 E	25.3 A	25.0 A	21.5 B	16.9 C	16.1 D	

*Means in the same column followed by the same letter are not significantly different at $p<0.05$

The highest number of nodules was obtained from chicken manure application in the same group with farm manure with 25.3 pieces / plant, while the lowest value was obtained from 8.9 pieces / plant and 0 kg/da (control) application. Table 4.22 in chickpeas in terms of Rhizobium bacteria vaccination. When examined, the highest number of nodules was obtained from the vaccination application with 21.4 units / plant, and the lowest value was obtained from the unvaccinated application (16.5 units / plant). Otieno et al. (2007), the results obtained from the studies of researchers are similar to the findings we have obtained. The highest value was determined as 28.4 numbers/plant from bacteria inoculation and chicken manure application. Chicken and farm manure were statistically included in the same group. In terms of the number of nodules, the lowest value was found as 7.4 numbers/plant in the non-grafted application.

CONCLUSION

The study was conducted to determine effects of *Rhizobium* inoculation traditional fertilization and different organic manures sources on the yield, quality and environment in chickpea in Fethiye ecological conditions. Experiment was laid out in randomized Complete Blocks Design with three replicates at the fields of farmer 2018 year in Kayaköy/Fethiye/Muğla. İnci chickpea cultivar was sown with, DAP (14 kg/da), chicken and farmyard manure (2 tones/da) manures, worm manure 300 kg/da, biohumus 150 kg/da and control. In the study were investigated the plant height, first pod height, branch number per plant, pod, seed number and per plant, seed number per pod, seed yield per unit, harvest index, 100-seed weight, number of nodule and protein ratio in seed. According to results of the study, while the highest seed yield per area was obtained from chicken, vermicompost, DAP, bio humus and farmyard manure application respectively, the lowest seed yield per area was obtained from control application. While the highest seed yield was obtained from chicken manure and rhizobium inoculation application with 200.3 kg/da, the lowest seed yield was obtained from uninoculation control parcels with 142.2 kg/da.

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CHAPTER 11

MICROORGANISMS INFECTING LENTIL (*Lens culinaris* Medik.) FOR ATMOSPHERIC NITROGEN FIXATION

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INTRODUCTION

Grain legumes are important food crops worldwide. They are among the ancient crops traditionally cultivated mainly in Asia, Africa and Latin America (Sarker et al., 2012). Lentil is among the oldest cultivated crop species. As a cool-season grain legume (pulse), dried seeds of this bushy and herbaceous plant are a good source of special healthy starches, proteins, dietary components, polyphenolics, trace elements for human diet. Apart from that, its straw is a good fodder for animals (Saxena, 2009; Salman and Hasan 2014; Shahwar et al., 2017).

Lens culinaris Medik. ssp. *culinaris* (Lentil), known for its resistance to cold and drought, is an ancient plant that originated from "*Lens culinaris* Medik. ssp. *orientalis"* in Asia Minor and Near East. (Biçer and Yılmaz, 2013; Laskar et al., 2019). This crop is a good food source of iron, protein, and energy in the human diet (Laskar et al., 2019). It is consumed as dried seeds (Bosmali et al., 2012). Lentil contains oligosaccharides from the raffinose family, resistant starches and some other prebiotic compounds required for maintenance of gastrointestinal microflora (Johnson et al., 2015). Lentils are rich in essential dietary components and trace elements (Thavarajah et al., 2011). Its sprouts also have the potential to be an effective dietary source of polyphenolics (Swieca et al., 2012). This bushy and herbaceous plant (Jameel et al., 2015) has considerable importance also as fodder (El-Nahas et al., 2011). Lentil straw is a good source of feed for livestock in South Asia, Africa and the Middle East. But straw traits improvement programs does not exist and straw traits do not consider new varieties release criteria (Alkhtib et al., 2017).

Lentil is an important food crop of developing countries (Sinha et al., 2019). It is usually grown in rainfed environments (Idrissi et al., 2015). Its drought tolerance is moderate, but terminal drought reduces lentil productivity significantly (Sinha et al., 2018). It is generally a part of a low-input cultivation system (Carbonaro et al., 2015).

Rhizobium leguminosarum is the usual symbionts of lentils. Whereas in different studies, *Rhizobium etli*, *Rhizobium laguerreae*, *Rhizobium leguminosarum* sv. *viciae, Rhizobium leguminosarum* sv. *trifolii, Mesorhizobium amorphae, Mesorhizobium huakuii* and *Ensifer numidicus,* were determined as inoculating lentils *(Lens culinaris*) (Rashid et al., 2014,2015).

Co-inoculation of cultivated lentil crop with *Azotobacter* (nitrogen-fixing soil bacteria), *Pseudomonas, Glomus intraradices* (mycorrhizal fungi) together with *Rhizobium* were also found beneficial in different studies (Desaii et al., 2016).

Fig. 1. Field view of lentil characterization program in “Indian Council of Agricultural Research / NBPGR” trials in India (Tripathi et al., 2020)

Variation in response to edaphic and climatic factors has resulted in the geographic differences and specific adaptation of lentil germplasm to its environment (Wiraguna et al., 2017). Lentil domestication location is in Eastern Mediterranean (Erskine et al., 2011). This species is an annual, diploid (2x=2n= 14), and self-pollinated plant. The history of lentil genetic improvement via breeding is not intense compared to cereals (Lombardi et al., 2014). Its genetic and genomic resources remained largely uncharacterized and unexploited (Mane et al., 2020).

MICROORGANISMS INFECTING LENTIL FOR ATMOSPHERIC NİTROGEN FIXATION

Rashid et al. (2012) conducted a phylogenetic analysis for genes of nodulation of lentil bacterial isolates from Syria, Turkey, Germany, and Bangladesh taken from lentil growth regions. They identified four distinct lineages of Rhizobia associated with lentil nodulation, three new and one endemic to Bangladesh, and one Central European and Mediterranean lentil symbiont (*Rhizobium leguminosarum*). Phylogenetic analysis of thirty-six bacterial isolates obtained from twenty-five locations in Bangladesh revealed that most of the isolates (30 isolates) were related to *R. leguminosarum* and *Rhizobium etli*.

In a study by Taha et al. (2018), the population structure and genetic diversity of 268 different symbiotic rhizobia obtained from 40 cultivated fields in Morocco were detected. Phylogeny of genes clustered >95% of the isolates into *Rhizobium laguerreae*. *Rhizobium laguerreae* was the main symbiont in Morocco for cultivated lentil.

48 lentil-nodulating rhizobia were isolated from the soil samples collected from diverse agro-ecological locations in Ethiopia by Tena et al., (2017) and characterized phenotype with 76 traits. Phenotypic characteristic analyses showed that the 48 tested strains were divided into three main clusters in *Rhizobium* genus. Genospecies II and Genospecies I were classified with *Rhizobium leguminosarum*, and *Rhizobium etli* respectively. Genospecies III was within the genus *Rhizobium* in an unnamed genospecies. The results confirmed the existence of Rhizobium species with lentil tuber with high variation.

Total 142 rhizobial bacteria were isolated by Dhaoui et al. (2016) from nodules of lentil (*Lens culinaris*) endemic to Tunisia from species *R. leguminosarum* and *Ensifer* (synonym: *Sinorhizobium*) and genera of *Mesorhizobium* never determined in lentil before. Based on 16S rRNA, rhizobia that nodulate lentil belonged to *R. leguminosarum* sv. *trifolii*, *R. leguminosarum* sv. *viciae*, *Mesorhizobium amorphae* (or M. loti), and *Ensifer numidicus* species. Based on these results, *Rhizobium leguminosarum* sv. *trifolii*, *Mesorhizobium* loti or *Mesorhizobium amorphae* sv. *ciceri* and *Ensifer numidicus* were proposed as new symbionts of cultivated lentils.

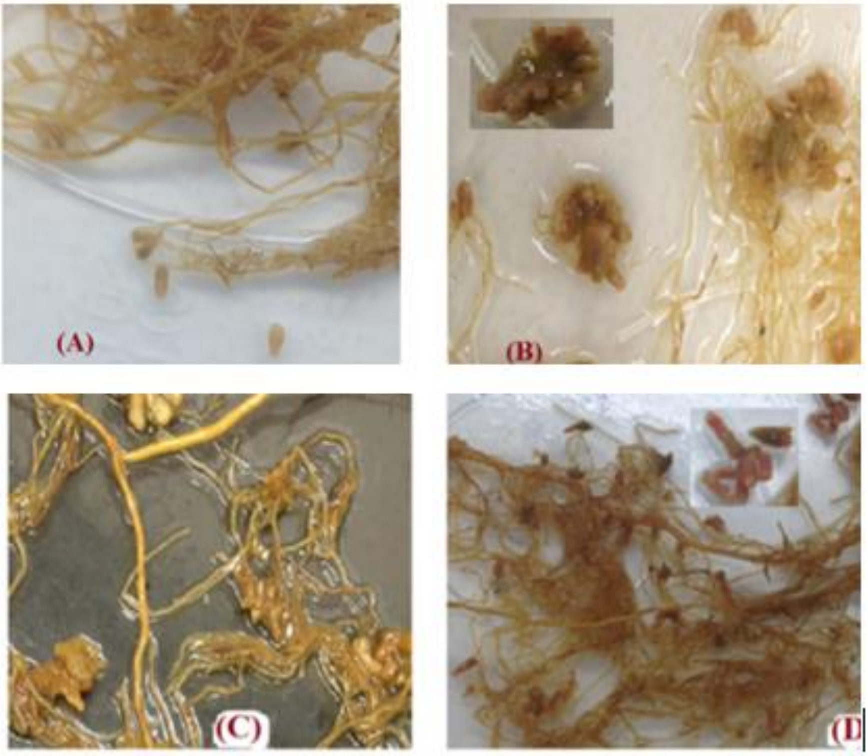

Fig. 2. Root nodules of cultivated and wild lentil genotypes inoculated with *Rhizobium leguminarum*. A) *Lens culinaris* B) *Lens orientalis* C) *Lens odemensis* D) *Lens lamottei* (Gorim & Vandenberg, 2017).

Amirnia et al. (2019) conducted field trials in Iran to see the effect of biofertilizers of N2 fixing bacteria (*Azotobacter*) and mycorrhizal fungi (*Glomus intraradices*) lentil under irrigated and rainfed conditions. Mycorrhiza + *Azotobacter* with supplemental irrigation increased biomass yield 37%, seed yield 28% and seed protein 38). The beneficial effect of mycorrhizal fungi + *Azotobacter* on photosynthesis, plant growth, and seed yield revealed the benefits of biofertilizers, especially under water-limited conditions.

In the research of Mishra et al. (2011), the effects of *Pseudomonas* and *Rhizobium leguminosarum* strains were examined on lentil growth. Bacterium combinations were found effective in nutrient uptake, plant development, and root system quality. Inoculation with *Pseudomonas* sp. strain promoted more vigorous vegetative growth, increased nodulation (55%), leghaemoglobin content (28%), total iron (91%), available iron (24%), chlorophyll content (44%), phosphorous uptake (37%) and nitrogen uptake (27%) sole *Rhizobium leguminosarum* determined at 60 days after sowing. Genetic and physiological adaptation of microbes to the environment was important. Variations for the efficacy of combinations revealed the specificity of the inoculums response. As a result, bacterial combinations was found suitable biofertilizers for lentil.

Sijilmassi et al. (2021) isolated total of 14 strains of *Rhizobium* from lentil accessions from ICARDA trial fields in Morocco and conducted symbiotic efficiency assessments and molecular characterization. Nodule number, plant dry weight, plant height and nitrogen content of leaves were determined. Phylogenetic analysis clustered *Rhizobium leguminosarum* and *Rhizobium laguerreae* with similarities ranging 94-100%. One exception was clustering one strain in *Mesorhizobium* *huakuii*. Bakria variety showed the best symbiotic efficiency with 10 strains compared to non-inoculated control.

Nodulation assays and cross-inoculation trials are required to determine the range of the host of lentil *Rhizobium* species. Nucleotide sequences of nodulation genes can be used to supply additional information for this aim (Santillana et al. 2008).

CONCLUSIONS

Rhizobium leguminosarum is the usual symbionts of lentils. Whereas in different studies, *Rhizobium etli*, *Rhizobium laguerreae*, *Rhizobium leguminosarum* sv. *trifolii*, *Rhizobium leguminosarum* sv. *viciae*, *Mesorhizobium huakuii*, *Mesorhizobium amorphae* and *Ensifer numidicus* were determined as inoculating lentils *(Lens culinaris*).

Co-inoculation of lentil crop with nitrogen-fixing soil bacteria (*Azotobacter*), *Pseudomonas*, mycorrhizal fungi (*Glomus intraradices*) and *Rhizobium* were found beneficial in different studies. Physiological and genetic adaptation of microbes to the natural environment is highly important.

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CHAPTER 12

COMMON WEEDS IN WHEAT FIELDS

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INTRODUCTION

Wheat, which is one of the basic nutrients of people, is the most cultivated crop in the world among cereal crops (Aktas et al., 2018; Cig et al., 2019). Wheat production is adversely affected by various abiotic and biotic factors. Weeds from the biotic factor compete with wheat for water, nutrients, light and space, causing significant economic losses in the yield and quality of wheat. Identification of weeds is important to prevent the loss of sources from weeds. Many grass and broad-leaved weeds are a problem in wheat fields. When the families of weeds that are a problem in wheat fields are examined, it is seen that *Asteraceae*, *Brassicaceae*, *Geraniaceae*, *Poaceae*, *Ranunculaceae*, and *Rubiaceae* families are common. When the wheat itself belongs to the Poaceae family, it can be said that it is difficult to control the weed species belonging to this family in general. Weeds should be controlled especially from the germination period of the wheat to the tillering period. In the early period, especially grass weeds may not be noticed when the field is not observed well. For this reason, field controls should be carried out regularly in the early periods after planting, and the covering areas and densities of weeds should be recorded. Wild oat (*Avena sterilis* L.), canarygrass (*Phalaris brachystachys* Link.), blackgrass (*Alopecurus myosuroides* Huds.), ryegrass (*Lolium multiflorum* L.), bluegrass (*Poa annua* L.), wild mustard (*Sinapis arvensis* L.), catchweed bedstraw (*Galium tricornutum* L.), corn buttercup (*Ranunculus arvensis* L.), cut-leaved crane’s-bill (*Geranium dissectum* L.), and Canada thistle [*Cirsium* *arvense* (L.) Scop.] species of weeds are common in wheat fields. Wild oat, canarygrass, blackgrass, ryegrass, and bluegrass species as grass, and wild mustard, catchweed bedstraw, corn buttercup, cut-leaved crane's bill, and Canada thistle species as broad-leaved weeds have higher incidence and densities.

Making digital herbariums of weeds and naming them in Latin will shed light on future studies. The preparation of catalogs on the biology and ecology of registered weeds will help to identify weeds and to predict periods when they may be a problem. Identifying these weed species at an early stage will help develop an appropriate control method. Weed control in wheat fields is carried out depending on herbicides in terms of being practical. grass weeds and broad-leaved weeds can be applied separately, or together with both effective herbicides or herbicide mixtures. Label information on herbicides should be read carefully. Consideration should be given to the miscibility of herbicides. If necessary, a preliminary jar test can be done to see the mixture visually. However, as a result of excessive and unconscious use of herbicides, herbicide resistance problems and some environmental problems occur in weeds. To prevent excessive herbicide use, weeds and economic damage thresholds should be known. Herbicides suitable for the problem weed should be used at the appropriate time and dose. Since it is known that weed control with only chemicals cannot be sustainable in the long term, cultural, mechanical and biological methods should be applied in an integrated manner. There is a need to develop integrated weed management specific to weed species for good agricultural practices and sustainable production in wheat.

1. WEEDS IN WHEAT

Weeds compete with wheat; they reduce the yield and quality of the crop by complicating agricultural maintenance works such as irrigation, fertilization and harvesting, and by hosting for pathogens and insects (Pala and Mennan, 2021). Weeds mixed with the crop at harvest can be carried to other areas or mixed with processed food and cause food poisoning (Bajwa et al., 2018; Pala et al., 2018). Weeds have a high germination and development rate, and also a high reproductive ability (Gardarin et al., 2011). If no protective treatment is applied to the cultivated plant, which is attacked by many weed species at once, even the crop may not be obtained from that field (Altland et al., 2003). In areas with known weed species densities, preventive measures, cultural measures, physical, mechanical, chemical and biological tactics from planting to harvest are trying to create an environment in which the cultivated plant can develop comfortably (Pala and Mennan, 2020b). Common weeds in wheat fields were summarized in Table 1.

Table 1. Common weeds in wheat fields

	Common Name	**Scientific Name**
1	Wild oat	*Avena sterilis* L.
2	Canarygrass	*Phalaris brachystachys* Link.
3	Blackgrass	*Alopecurus myosuroides* Huds.
4	Ryegrass	*Lolium multiflorum* L.
5	Bluegrass	*Poa annua* L.
6	Wild mustard	*Sinapis arvensis* L.
7	Catchweed bedstraw	*Galium tricornutum* L.
8	Corn buttercup	*Ranunculus arvensis* L.
9	Cut-leaved crane's-bill	*Geranium dissectum* L.
10	Canada thistle	*Cirsium arvense* (L.) Scop.

(Pala and Mennan, 2017a).

Weeds that are a problem in wheat fields are generally planted specialized for cereals (Arslan et al., 2017; Arslan, 2018: Pala et al., 2020a). Even if grass wild oats are four plants per square meter (Kadioglu et al., 1998), broad-leaved wild mustard is about one plant per square meter (Mennan, 2003), the control should be started. Weed control in wheat can be done with chemical methods, from the 3-leaf period of the wheat to the end of tillering (Pala and Mennan, 2021). Applications made after this period may not have the desired effect on weeds or may damage the wheat plant (Pala and Mennan, 2017b). Yield losses can be prevented as a result of the biological effect of herbicide use on weeds, but it should not be ignored that herbicides may also hurt the crop (Safi, 2016). In recent years, pre-emergence herbicides have also been used against the grass and broad-leaved weeds in bread wheat (Agronomy eUpdates, 2021). Herbicide options applied at different times are important in terms of herbicide rotation. Of course, the main point to be considered here is the preference for herbicides with different action sites or mechanisms. In addition to these, bioherbicides or allepatic extracts can be developed as an alternative tactic against weeds in wheat (Safi & Al-Faid, 2018).

One of the methods that have come to the fore in the diagnosis or control of weeds in recent years is sensitive sensors, artificial intelligence and robotic technology. In our changing and transforming world, there is a need for research, development and cooperation of new technologies to reduce yield loss in wheat, which is one of the basic nutrients of people for adequate and healthy food supply to humanity.

1.1. Wild oat (*Avena sterilis*)

Figure 1. Wild oat (Bayer, 2021; Monte Pallano, 2021)

Wild oats are in the grass (*Poaceae*) family (Sebastin et al., 2018). It is an annual, most seeds germinate in the spring, the seed is awned, true leaves develop counterclockwise. Ligule membranous, long rounded or slightly toothed, auricles are absent. Blade is rough, with sparse, long hairs on the leaf margins at the base, mostly. It is fringe rooted. The flowers are in the form of clusters. The inflorescences each have nodding spikelets from 2-3 flowers (cluster). The seed is awn (Agrobaseapp, 2021; Bayer, 2021). Wild oat is an important problem, especially in cereal fields. It has become more trouble in monoculture areas than crop rotation fields.

1.2. Annual canary grass (*Phalaris brachystachys*)

Figure 2. Canary grass (Bayer, 2021; Monte Pallano, 2021)

Annual canarygrass is in the grass (*Poaceae*). It is an annual herb. This species does not have auricles, ligule is short, torn-light hairy (Agrobaseapp, 2021; Bayer, 2021). Meanwhile, *P. canariensis* is a domesticated form of *P. Brachystachys* (Oram, 2004). This short-capped species can be confused with the long-capped and soft-capped species of the same genus in terms of similarity. Although it is more common at the edges of the fields, a significant problem may occur in some wheat fields. Like other grass weeds, necessary preventive measures should be taken, cultural treatments should be done and herbicides should be used if necessary to keep this species below the economic damage threshold.

1.3. Black grass (*Alopecurus myosuroides*)

Figure 3. Black grass (Bayer, 2021; Monte Pallano, 2021)

Blackgrass is in the grass (*Poaceae*) family. It is an annual plant. Blackgrass is a winter plant in the same family as wheat. This species' auricles are absent, ligule is medium finely serrated. There are overlapping margins, but this is not a flap (Agrobaseapp, 2021; Bayer, 2021). It is the problem of grain fields, especially wheat. In recent years, studies have been carried out on the determination of morphological and molecular species due to herbicide resistance problems (Uludag et al., 2003). It is more intense on the field edges. Chemical control is preferred against this weed in wheat fields.

1.4. Italian ryegrass (*Lolium multiflorum*)

Figure 4. Ryegrass (Bayer, 2021; Monte Pallano, 2021)

Annual ryegrass is in the grass (*Poaceae*) family. It is an annual herb. It is a winter plant in the same family as wheat. It is annual and fringe rooted. The plant grows upright. Its leaves are shiny. The spike consists of alternately ordered spikelets. The ligule is usually small, membranous, and somewhat pointed at the apex. The auricles are small arm-like projections that attach to the junction of the blade and sheath (Agrobaseapp, 2021; Bayer, 2021). *Lolium multiflorum* is called annual grass or Italian grass. *Lolium perenne* is known as perennial grass or English grass or turf (Hannaway et al., 1999). It is commonly controlled with herbicides.

1.5. Bluegrass (*Poa annua*)

Figure 5. Bluegrass (Bayer, 2021; Monte Pallano, 2021)

Bluegrass is in the grass (Poaceae) family. P. annua is an annual, P. Pratensis and P. Trivialis perennial (Carroll et al., 2021; Purdue-Extension, 2021; UC-IPM, 2021). Bluegrass is a winter plant in the same family as wheat. It tends to grow semi-slant. The leaves are boat-shaped. The ligule is prominent in the membranous shape (Agrobaseapp, 2021; Bayer, 2021). There are no auricles. Poa species are also used in grass mixes such as Lolium and Festuca. However, it can be a problem in wheat fields as a weed. Bluegrass in wheat is controlled with herbicides used in the post-emergence tillering period.

1.6. Wild mustard (*Sinapis arvensis*)

Figure 6. Wild mustard (Bayer, 2021; Monte Pallano, 2021)

Wild mustard is in the mustard (*Brassicaceae*) family. It is an annual plant. It forms rosette leaves during the germination period (Agrobaseapp, 2021; Bayer, 2021). Composed of four petals, the yellow flowers develop into a carob-shaped pod, and each pod contains about 10 seeds. Wild mustard has a high ability to reproduce, spread and adapt. It is important to know the biology of this broad-leaved weed, which is common in wheat fields (Atas et al., 2017; Atas and Uremis, 2019). Wild mustard should be controlled even if there is only one per square meter. Wild mustard control in wheat fields is carried out depending on herbicides. Therefore, it is known that the herbicide resistance problem is increasing in wild mustard.

1.7. Catchweed bedstraw (*Galium tricornutum*)

Figure 7. Catchweed (Bayer, 2021; Monte Pallano, 2021)

Catchweed bedstraw is in the madder (*Rubiaceae*) family. It is an annual plant. It usually clings or adheres to crops (Buyukkurt and Uludag, 2019). The first true leaves are elliptical or narrow, with spiny ends, and arranged in four or more spoke-like folds. It has hairs clustered near leaf spirals that cause the weed to cling to neighbors. The leaves are in rounds of eight, sometimes six, and have a strong central vein and an indeterminate stem. Leaf margins and midribs are jagged and leaf tips are spiny. Pile rooted. Individually stem flowers are grouped in clusters originating from the leaf axils. The flowers are four-petaled. The fruits are divided into two identical parts, each containing a seed (Agrobaseapp, 2021; Bayer, 2021).

1.8. Corn buttercup (*Ranunculus arvensis*)

Figure 8. Corn buttercup (Bayer, 2021; Monte Pallano, 2021)

Corn buttercup is in the buttercup (Ranunculaceae) family. They are large (up to almost a centimetre long - quite a size for such a small plant), oval and covered in spines up to 2 mm in length. Corn buttercup leaves are stalked and deeply dissected, divided into 3-5 lobes (Agrobaseapp, 2021; Bayer, 2021). It is an important weed problem in wheat and lentil rotation systems (Pala and Mennan, 2018). It is controlled with postemergence herbicide application.

1.9. Cut-leaved crane's-bill (*Geranium dissectum*)

Figure 9. Cut-leaved crane's-bill (Bayer, 2021; Monte Pallano, 2021)

Cut-leaved crane’s-bill is in the geranium (*Geraniaceae*) family. It is increasingly being made in many habitats from the Mediterranean region to the Himalayas (Aedo et al., 2005). In dove's foot, the first and subsequent true leaves are not as deeply dissected as in cut-leaved. It has asymmetric cotyledons, long stems with conspicuous hairs and palmate leaves (like a sycamore tree), mature plant showing the deeply divided leaves, and small pinkish-violet flowers with deeply incised petals (Agrobaseapp, 2021; Bayer, 2021). This weed species, which is a problem in wheat, is tried to be controlled with herbicides.

1.10. Canada thistle (*Cirsium arvense*)

Figure 10. Canada thistle (Bayer, 2021; Monte Pallano, 2021)

Canada thistle is in the sunflower (*Asteraceae*) family. It is a perennial herb with storage roots and rosette forming (Ucrak and Gurbuz, 2018). Mature plants can be up to 1.5 m tall and have a branched structure towards the top. The stems are hollow, grooved and sparsely hairy. The leaves are 5-15 cm long, alternate, oblong and irregularly lobed with sharp spines on the margins. The upper surfaces of the leaves are hairless, dark green and waxy, while the lower surfaces are pale green and smooth, and the leaf margins are spiny. The leaves are sessile and pinch the stem from the base. Male and female flowers are produced on separate plants at the leaf axils and branch tips (Agrobaseapp, 2021; Bayer, 2021). Many thistle and thistle-like plants have spiny leaves and flower heads similar to Canadian thistles.

CONCLUSION

It should be noted that knowing weeds is the first step in developing appropriate control strategies. There are dozens of different weed families and hundreds of species that are a problem in wheat fields. However, if the ten weed species mentioned above are well known and integrated weed management is implemented, this problem can be brought under control.

Tall wheat varieties can be preferred because they are more competitive against weeds that are a problem in wheat fields, if winter planting is done, the competitiveness of wheat can be increased, early germinated weeds can be removed by cultivator by late planting, weed germination or development can be prevented by frequent planting, In the period from post-harvest to pre-planting, the soil can be overturned with a plow and the germination feature of weeds that will germinate that year can be lost.

Winter wheat is usually planted in October-November, and some of the weeds that are a problem germinate together with wheat, and some germinate in February-March. Weed competition increases with the germination of weeds in the spring. Mechanical control of weeds during the vegetative growth period of wheat is very limited. For this reason, the use of herbicides is preferred in the control of weeds in wheat. Continuing the control of such weeds, which is a problem related to herbicides in wheat, has caused resistance to herbicides in some species, especially wild oats and wild mustard. Integrated weed control should be included in good agricultural practices to delay resistance formation.

The management of weed species and density needs to pay attention to the following seven issues related to weeds that are a problem in wheat fields:

1) Field-based weed records should be kept.

2) Existing weeds should be determined.

3) Weed catalog should be prepared.

4) Weed map of the field should be drawn.

5) The biology and ecology of the problematic weeds should be learned.

6) Weed species-specific management strategies should be developed.

7) Integrated weed control should be done according to good agricultural practices.

Finally, it should not be forgotten that modern and alternative methods such as developing clearfield wheat varieties have been included in the weed problem in wheat in recent years.

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