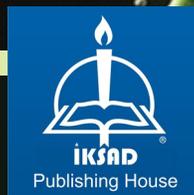


Grain Legumes Under Abiotic Stress: Yield, Quality, Enhancement and Acclimatization

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Prof. Dr. Murat ERMAN



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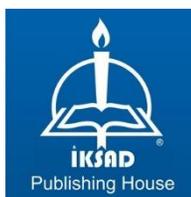
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PREFACE

Legumes represent the most utilized plant family with 20,000 species and are among the most important crops worldwide, having major impacts on agriculture, the environment, and human/animal nutrition and health. Out of these legume species, grain legumes rank third behind cereals and oilseeds in World production, accounting for 27% of the world's primary crop production. Grain legumes have a pivotal role as a dietary constituent for humans and animals and these alone contribute 33% of the dietary protein nitrogen (N) needs of humans besides being a source of income and livestock feed. Legumes are often exposed to environmental biotic and abiotic stresses that reduce productivity throughout the world. Particular abiotic stresses such as salt, drought, temperature, UV, nutrient deficiency and heavy metal are responsible for more than 50% yield reductions of some major crops. Thus, understanding of stress mechanisms and responses of legume plants provides advantages in stress management, mitigation of adverse stress impacts and leads to improve stress tolerance.

This book is a comprehensive account of current knowledge about the physiological responses and adaptability of legumes to abiotic environmental stresses, and some new approaches to stress management. We have tried our best to ensure the accuracy of the information in this original and current study. We are also very grateful to the authors for their valuable contributions and the IKSAD publication house.

Prof. Dr. Murat ERMAN

CHAPTER 1

THE BENEFITS OF LEGUMES FOR AGRICULTURE SUSTAINABILITY

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INTRODUCTION

Nowadays, with the increasing food demand in parallel with the rapid increase in the world population, concerns about the environment and food security are increasing (Rubiales and Mikic, 2015). On the other hand, the continuous sequential cultivation of the same products in agricultural areas, which are the main source of food raw materials, and the continuous exploitation of the soil by the same plants cause the acceleration of soil erosion, the depletion of soil nutrient reserves, and the deterioration of the physical, chemical, and biological properties of soils. Therefore, agricultural practices should be developed to ensure food security and meet the increasing demand for food while minimizing environmental impact. To this end, there is a need more than ever for minimizing agricultural inputs, improving biodiversity, diversifying the cropping system, and using legumes extensively in crop rotation systems, and implementing a simple but effective and economical set of measures and/or agricultural practices that improve soil fertility.

The crops grown in many parts of the world and the agricultural systems applied have strategic importance for the future of living beings. For example, the rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) agricultural production system in Asian countries (Nath et al., 2019), sweet maize (*Zea mays* L. *saccharata*) production in China (Wang et al., 2021) and cereal-based rotation systems such as wheat-corn-wheat in Pakistan (Arif et al., 2021) contribute significantly to the world food and cereal production share, and therefore the sustainability of these

systems is important for food security. However, nowadays, the sustainability of these cereal production systems and other similar systems is under threat due to many reasons, especially the decrease in underground water resources, unconscious fertilization and irrigation, intensive tillage, burning of harvest residues, depletion of soil organic carbon (SOC) reserves, and deterioration of soils. Additionally, the decrease in crop productivity due to the low soil organic matter (SOM) in many developing countries (Ozyazici and Turan, 2021) is also remarkable.

Therefore, soil management and plant production techniques that ensure soil health should be improved and developed to sustain the productivity-yielding power of the soils and the yield capacity of crops in the long term. In this sense, agricultural practices such as diversifying and designing plant production systems, including legumes, and increasing crop diversity, come to the forefront. This review article examined the effects of legumes on soil fertility and crops yield and quality in terms of agricultural sustainability.

BIOLOGICAL NITROGEN FIXATION AND SUSTAINABILITY OF CROPPING SYSTEMS INCLUDING LEGUMES

Nitrogen (N) is an essential plant nutrient required to increase the yield of many important agricultural products (Jnawali et al., 2015; Özyazıcı, 2020a) and is an important factor limiting plant production in case of its deficiency (Mapfumo, 2011; Dong et al., 2012; Santi et al., 2013; Özyazıcı, 2020b). For this reason, it is a fact that the amount of nitrogen

required for agriculture will increase as the demand for agricultural products will increase, especially in parallel with the increase in drought worldwide. However, this may lead to excessive and unconscious use of fertilizers in agricultural lands and may also pose a threat to the sustainability of natural resources. Thus, paying more attention and including agricultural practices that reduce dependence on N fertilizers and support biological nitrogen fixation will provide significant benefits to both agriculture and the environment (Olivares et al., 2013). Biological nitrogen fixation is considered an alternative solution for cost-effective agricultural production for many farmers experiencing economic difficulties (Mafongoya et al., 2006). In terms of biological nitrogen fixation, legumes with the highest quantitative effect on the N cycle and symbiotic systems in which *Rhizobium* bacteria play a role come to the fore; symbiotically fixed N represents a renewable source of N in agriculture (Mohammadi et al., 2012). Legumes (Fabaceae), a family of dicotyledonous, are important food products worldwide due to their high N content as a result of symbiosis with *Rhizobium* bacteria (Dita et al., 2006; Bayraklı et al., 2017). In this sense, the presence of legumes, which play a role in biological nitrogen fixation, in agricultural systems, either in single crop rotation systems or in the form of intercropping with other products, will increase the sustainability of agricultural production and will also make a significant contribution to sustainable soil management strategies.

The Importance of Legumes for Sustainable Soil Fertility

Traditional soil fertility management methods make the increasing food demands of the world population significantly inadequate. Therefore, integrated soil fertility management (ISFM) approaches gain importance (Mapfumo, 2011; Pypers et al., 2011). Appropriate land use and soil management practices, which especially serve to improve and protect SOM, have important effects on the preservation and increase of soil fertility (Whitbread et al., 2003; Puget and Lal, 2005). The most common definition of soil fertility is the ability of a soil to provide nutrients to crops (Watson et al., 2002). Within the framework of this approach, legume plants have significant advantages in improving soil fertility, such as increasing SOM, improving soil porosity, recycling plant nutrients, making soil pH, which is an important chemical component of the soil, suitable for plant nutrition, and increasing microorganism activities in the soil. Intercropping systems with legumes and crop rotation systems including legumes improve the physical, chemical, and biological properties of soils and are effective in sustaining and improving soil fertility.

The role of legumes in the physical quality of soils

Plant production and soil health are strongly affected by the physical quality of the soil, especially in the 0-20 cm root zone of the soil profile, which is considered the fertility layer. Plant production systems, including legumes, play a significant role in improving the physical properties of soils. This effect of legumes originates from the fact that root and stubble residues have a significant value as a source of organic

matter. Aranyos et al. (2016) reported that organic matter plays an important role in the aggregation of soil particles, as a result of which soil compaction decreases, promoting water infiltration in the soil and thus maintaining/improving the physical properties of the soil.

A study conducted in the arid conditions of the Eastern Anatolia Region of Turkey (Hanay et al., 1998) determined that crop rotation systems including legume forage crops such as vetch and sainfoin improved the physical properties of soils and improved soil-water relationships. Similar results were also observed in the lentil-wheat rotation system applied in the conditions of the Central Anatolia Region of Turkey, and it was reported that soils became more suitable in terms of the total porosity volume (Adak et al., 1998) and infiltration rate (Eser et al., 1998) after lentils. Latif et al. (1992) reported that the intercropping system of alfalfa, clover and hairy vetch with corn (*Z. mays* L.) had significant and positive effects on some physical properties of soils, the lowest stability of soil aggregates and the smallest mean weight diameter were obtained from the area cultivated with monoculture corn. The researchers emphasized that these positive effects in intercropping were a result of green manure values of legumes depending on increased root activity.

Feiziene et al. (2016) reported that the best sustainable soil layer was determined in the crop rotation, including red clover (*Trifolium pratense* L.), and the best soil quality was obtained as a result of diversifying crop rotations with the inclusion of legumes in the cereal rotation. The same researchers revealed that this rotation, in which the

highest soil mesoporosity and the lowest microporosity were obtained, was the best supplier of plant-available water and also had high soil resistance to dry conditions. Furthermore, the researchers stated that non-legume rotations did not contribute to soil water conservation and soil respiration.

Soil water retention is an important soil hydraulic feature that manages soil functioning in ecosystems and considerably affects soil management (Rawls et al., 2003). Soil texture and organic matter are the basic components of the soil that determine the water holding capacity (WHC) of soils (Rajpoot et al., 2021). Rawls et al. (2003) reported that the SOM content and composition affected both soil structure and adsorption properties, therefore, changes in SOM, affected by both climate change and changes in management practices, might affect the water retention properties of soils. The same researchers reported that the effect of changes in the organic carbon (C) content on soil water retention depended on the ratio of soil texture components and the amount of organic carbon in the soil; in this sense, at low carbon contents, an increase in carbon content led to an increase in water retention in coarse-textured soils and a decrease in fine-textured soils; in contrast, at higher carbon contents, an increase in carbon content caused an increase in water retention of all textures. Rajpoot et al. (2021) reported that the WHC of soils under the rice-chickpea rotation system was higher than that of soils under rice-wheat, rice-field pea, rice-mustard, rice-linseed and rice-fallow systems. In the same study, it was determined that the WHC value of rice-field pea

system soils was higher compared to rice-linseed and rice-fallow systems. In light of these findings in the literature, it can be said that legumes, which play a role in the increase of organic matter in soils, have a significant effect on the conservation/retention of soil water.

Bulk density is one of the important physical properties of soils that determine the porosity, aggregate stability, and WHC of soils and root development (Rajpoot et al., 2021). Rajpoot et al. (2021) determined that soils under rice-legume rotation systems (rice-chickpea and rice-field pea) had lower bulk density values than other systems (rice-wheat, rice-mustard, rice-linseed, rice-fallow). The researchers stated that the high amount of biomass added to the soil with legumes loosened the soil, increased porosity and reduced soil compaction, which was effective in lowering the bulk density values of the soil in rice-legume rotation systems. Similar findings were also reported by Rahman et al. (2007) and Kumar et al. (2018a, 2020).

A study examining the effect of leguminous trees (*Senna siamea*, *Gliricidia sepium* and *Leucaena leucocephala*) on the physico-chemical properties of an Alfisol revealed that legume species improved the texture class of the soil from loamy sand to sandy loam at a soil depth of 0-15 cm and bulk density values increased throughout the soil depth in soils under leguminous tree species, compared to natural fallow areas (Olujobi, 2016).

In a study conducted with different legumes, lower soil bulk density values were determined under the potato-legume intercropping system compared to the system cultivating sole potatoes. In the same study, it

was reported that potato-legume intercropping systems would contribute to the sustainable improvement of soil fertility over time (Gitari et al., 2019).

Soil porosity is the best indicator of soil structural quality and is an effective parameter in understanding the soil changes caused by agricultural management practices (Rajpoot et al., 2021). Soil porosity values may vary in soils under different cropping systems and different crop rotation systems. Rajpoot et al. (2021) reported that the porosity of soils under the rice-chickpea rotation system was higher than that of soils under rice-wheat, rice-mustard, rice-linseed, and rice-fallow. The fact that legume-based cropping systems have higher carbon sequestration capacity than other cropping systems makes the soil looser and more porous (Kumar et al., 2019, 2020).

Soil moisture is another important factor affecting the sustainable cropping system (Rajpoot et al., 2021). Therefore, nowadays, it is important to implement plant cultivation systems that minimize moisture loss in the soil and maintain moisture for the longest time in agricultural areas under the threat of climate change and global warming. In this sense, agricultural systems, including legumes, come to the fore. In their study, Rajpoot et al. (2021) determined that the moisture content of soils under rice-legume rotation systems was much higher than in systems without legumes. Similar findings were also reported by Rahman et al. (2007), Alam and Salahin (2013), and Kumar et al. (2020). Likewise, intercropping systems with legumes (especially cereal-legume) prevent evaporation losses by creating more cover on

the soil surface. Moreover, water use efficiency increases since plants that make up the intercropping system benefit from the water in different soil horizons through their roots going to different soil depths (Sani et al., 2011; Iqbal et al., 2019).

To improve soil quality, cover crops can be included in the cropping system (Demir et al., 2019a). The use of legumes as cover crops increases SOM more than other cover crops (Teodoro et al., 2011, Demir et al., 2019a). Researchers emphasized that legumes improved the physical properties of soils owing to their positive properties mentioned. Thus, Demir et al. (2019a) reported that in case of using legume forage crops (*Vicia villosa* and *Vicia pannonica*) as cover crops in an apricot orchard, the highest field capacity, permanent wilting point, available water capacity, total porosity, aggregate stability, and structural stability index values were obtained in comparison with other cover crops. In the same study, it was determined that depending on the accumulation of organic matter, the bulk density values were lower in soils under the legume cover crops compared to other cover crops. Similar findings were also found in the soils of persimmon (Demir and Işık, 2019a), kiwifruit (Demir and Isik, 2019) and hazelnut (Demir and Isik, 2020) orchards where *Trifolium repens* L. and *Vicia villosa* Roth. were used as cover crops.

Some other studies have also reported that soil and water losses decreased (Zhu and Zhu, 2012; Prosdocimi et al., 2016) and the aggregate stability of the soil improved (Ramos et al., 2011) with the cultivation of legume forage crops as intermediate or cover crops in

orchards. Again, in a study using hairy vetch as a cover crop, it was revealed that the infiltration rate was higher compared to fallow areas (McVay et al., 1989).

Legume root and harvest residues, which are rich in nitrogen, form living environments for many micro-organisms and living things in the soil. This environment increases soil porosity and promotes soil aeration, air circulation, and water infiltration. A large amount of biomass is added to the soil in all plant cultivation systems including legumes, and these systems also store large amounts of carbon in the soil. All these general characteristics of both grain legumes and legume forage crops are accepted as the main reason for the improvement of the physical structure of soils.

The role of legumes in the soil chemical quality

Soil organic carbon is the main indicator of soil health and quality and plays an important role in the sustainability of soils (Abid and Lal, 2008; Kumar et al., 2018b). SOC is the main component of SOM and has a very important function in improving the physical, chemical, and biological properties of the soil and increasing the productivity of crops (Alagöz and Yilmaz, 2009; Yang et al., 2009, 2012). Many factors such as climatic factors, soil type, cropping system, and crop residue are effective on SOC sequestration (Lou et al., 2011; Zhu et al., 2015).

In general, crop rotation systems increase SOC and organic matter content and improve agricultural sustainability by helping to maintain soil structure and quality (VandenBygaart et al., 2003; Liu et al., 2006;

Lal, 2016; McDaniel and Grandy, 2016). Considering that legumes have a potentially important role in increasing soil carbon sequestration (Abberton, 2010), the inclusion of legumes in crop rotation can increase the total amount of SOC (Shah et al., 2003, 2011; Ghimire et al., 2017; Rajpoot et al., 2021). Kumar et al. (2018b) stated that legumes have the capacity to store 30% more SOC compared to other species, owing to their biological nitrogen fixation ability, and that legumes contribute directly to the SOC pool, both above and below the ground. Therefore, an important part of the SOC pool is production areas where legume agriculture is carried out.

Blair and Crocker (2000) reported that including legumes in the rotation increased soil carbon concentration compared to continuous wheat or a long fallow period. Lynch et al. (2005) stated that overseeding with legumes in pastures might cause an increase in SOC, and Zhang et al. (2009) expressed that the conversion of reed meadows to alfalfa fields might cause an increase in SOC. Mechri et al. (2016) reported that fallow fields maintained the original SOC content (0.79%) throughout the entire production season, they were followed by faba bean species, there was lower SOC in areas cultivated with wheat and barley compared to fallow fields, and faba bean left more organic matter in the soil compared to cereal species. The researchers reported that the low SOC value in cereal fields might originate from the monoculture production system in the form of a cereal-cereal system.

It was reported that including legumes in crop rotation systems with cereals improved soil quality, and in this sense, the highest soil organic

C sequestration, an increase in soil N and potassium (K) reserves, and the highest soil net CO₂ exchange rate were observed in cereal rotations containing red clover (*Trifolium pratense* L.). In the same study, it was stated that non-legume rotations did not contribute to soil agrochemicals, there was a loss of large amounts of macronutrients in the soil of these rotations, they caused a 26-33% lower soil net CO₂ exchange rate compared to legume rotations, and non-legume rotations did not support the sustainability of soils (Feiziene et al., 2016). Studies carried out in orchards reported that higher SOC stocks and SOM content were determined with the cultivation of legume forage crops as intermediate or cover crops (Ramos et al., 2011; Liu et al., 2013; Zhong et al., 2018).

A study examining the effects of continuous corn (*Z. mays* L.), corn (*Z. mays* L.) and wheat (*Triticum aestivum* L.) rotation system and 9-year cereal-alfalfa [corn-wheat-corn-wheat-corn-wheat-alfalfa-alfalfa-alfalfa (*Medicago sativa* L.)] crop rotation systems on some soil properties determined that MBC (microbial biomass carbon), SOC, total N and MBN (microbial biomass nitrogen) values increased by 37%, 24%, 23%, and 23%, respectively, with cereal-alfalfa rotation in comparison with other rotations. The same study stated that long-term rotations, including legume forage crops, could be accepted as a sustainable agricultural practice in terms of both soil quality and crop productivity (Giacometti et al., 2021). Many other research findings (Odunze, 2003; Fornara and Tilman, 2008; Ogunwole et al., 2010; Adesoji et al., 2014; Meena et al., 2018; Oliveira et al., 2019; Borase et

al., 2020) also stated that SOC increased as a result of mixing legumes into the soil and/or in soils where legumes were cultivated and legumes could promote carbon storage.

Legumes, which have the ability to fix the free nitrogen of the air to the soil through the Rhizobium bacteria in their roots, thus ensure that both the organic residues in the soil and the harvest residues of the following crops in rotation are easily broken down in the soil and these residues pass into the soil structure. This naturally causes an increase in SOM. Soil organic matter ensures that nutrients in the soil become useful to the plant. Additionally, legumes enable the plant nutrients in the soil profile to be converted into forms that can be taken by plants through their tap-roots that can go to different soil depths.

Giller (2001) stated that legume-cereal rotation systems, provide protein-rich cereals for human nutrition, on the one hand, and contribute to the increase in soil fertility by fixing atmospheric N, on the other hand. Adak et al. (1998), Jensen et al. (2012), and Das et al. (2018) indicated that cropping systems including legumes improve soil fertility by increasing the humus content of soils, N and phosphorus (P) availability.

It was observed that the organic matter, organic C and total N values of the soils at the end of the corn and wheat harvest were usually higher in the forage faba bean+corn+wheat crop rotation system compared to the fallow+corn+wheat system (Özyazıcı and Özdemir, 2013). A study in which biochar, green manuring, chemical fertilization and legume-rice rotation systems were applied and mucuna (*Mucuna pruriens*) and

cowpea (*Vigna unguiculata*) were used as leguminous plants reported that application issues involving legumes (such as legumes alone, biochar x legumes, legumes x fertilization) increased the inorganic N, available P and cation exchange capacity (CEC) of soils (Partey et al., 2016). Uzoh et al. (2019) reported that legume-corn rotation systems increased the total N, organic C, available K and magnesium (Mg) content and CEC of soils compared to the corn-corn system. Rajpoot et al. (2021) determined that soils under rice-legume rotation systems consisting of rice-chickpea and rice-field pea had higher available N, P, K, iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), and boron (B) content than soils of rice-wheat, rice-mustard, rice-linseed, and rice-fallow systems.

Intercropping systems of cereals-legumes improve nutrient utilization because different plants have different root lengths, thereby absorb nutrients from different soil horizons (Hailu and Geremu, 2021).

It was reported that the corn (*Z. mays* L.) and cowpea (*Vigna unguiculata* L.) intercropping system increased the N, P, and K content of soils compared to the sole corn cropping system, the highest N, P, and K amounts were identified in the soil in the sowing of cowpea alone and in mixed cowpea-corn sowings with the highest ratio of cowpea, and intercropping systems were effective in maintaining soil fertility compared to sole corn cultivation (Dahmardeh et al., 2010).

A study conducted in an agroforestry area consisting of legume tree species (*S. siamea*, *G. sepium*, and *Leucaena leucocephala*) determined

that the pH, N, and P values were significantly higher in soils under legume tree species compared to natural fallow fields (Olujobi, 2016).

It was revealed that potato-legume intercropping systems including *Lablab purpureus* L., *Pisum sativum* L. and *Phaseolus vulgaris* L. caused significant improvements in the soil pH and significant increases in the available P, total N, organic C and CEC values of soils compared to systems in which sole potato was cultivated. The same study reported that the potato-legume system containing *Lablab purpureus* L. contributed to the sustainability of the soil (Gitari et al., 2019).

Soil properties such as soil texture, moisture content, pH, and available N have significant effects on the degradation of organic matter (Jarvis et al., 1996). This also causes significant changes in soil pH (Tang and Yu, 1999). Low pH reduces microbial activity and decomposition of organic matter (Motavalli et al., 1995; Adeboye et al., 2006). Therefore, an appropriate level of soil pH in plant production is of great importance both in terms of soil fertility and plant yield and quality. Cultivating legumes in areas where the soil pH value is a problem will contribute to increasing the organic matter content of the soil and providing a suitable environment for microbial activity in the soil. However, considering that the direction and magnitude of change in soil pH largely depend on the organic anion concentration of the materials mixed into the soil, the initial soil pH, and the degree of decomposition of the residue (Tang and Yu, 1999), it should not be expected that changes in soil pH are sudden and short-term. In this sense, it is

recommended to add leguminous plant residues with high cation content to the soil to minimize soil acidification in agricultural systems (Tang and Yu, 1999). Thus, a study (Adeboye et al., 2006) determined that the crop rotation system significantly affected the soil pH, total N and organic C amount compared to fallow fields and that the soils under crop rotation, including *Centrosema pascuorum* legume, had the highest pH value, significantly differing from other rotated soils.

The use of especially legume forage crops as cover crops plays a major role in increasing the amount of some macro and micronutrients in soils, especially organic matter, and this plant cultivation system makes significant contributions to the sustainability of soil and efficient crop management.

A study in which a mixture of *Vicia villosa* and *Vicia pannonica* and *Vicia pannonica* (70%) + triticale (30%) among legume forage crops in an apricot orchard was addressed as cover crops revealed that compared to the control, SOM, total N, available P, extractable K, calcium (Ca), and Mg values increased and pH and exchangeable sodium (Na) contents decreased at a 0-20 cm soil depth with cover crop applications (Demir et al., 2019a). Similar findings were also found in a study using legume forage crops as cover crops in kiwifruit (Demir and Isik, 2019), hazelnut (Demir and Isik, 2020) and cherry (Demir, 2020) orchards. A study using *Trifolium repens* L. and *Vicia villosa* Roth. as cover crops reported that the highest organic matter value was obtained in the soils (Demir and Işık, 2019a). Likewise, Ramos et al. (2011) indicated that

legume cover in orchards significantly increased the amount of total N and available K.

A study carried out in a clay-textured apricot orchard in Malatya province, Turkey, determined the highest Fe, Mn, and Zn contents of the soils in the application with *Vicia villosa* Roth. as a cover crop. The same study concluded that especially *Vicia villosa* Roth. and *Vicia pannonica* Crantz applications as cover crops could be included in the cropping system to increase micronutrients and ensure sustainable soil management (Demir et al., 2019b). Similar findings in terms of microelements were also reported in practices in which legume forage crops were cultivated as cover crops in hazelnut and apple orchards (Demir and Işık, 2019b).

The role of legumes in improving the biological properties of soils

The soil enzyme is one of the main components of the soil (Li, 1981), and soil enzyme activity is an important part of the biochemical process that plays a role in soil productivity (Shun and Tong, 2001) and an indicator of soil quality (Gianfreda et al., 2005). Soil microorganisms and the microbial activity of soils play an important role in the nutrient cycle and conversion, organic matter decomposition, and the formation of a stable soil structure (Tamilselvi et al., 2015; Adetunji et al., 2017). Furthermore, soil microorganisms mediate many agriculturally beneficial processes such as nitrogen fixation, recycling of nutrients such as N, P and sulfur (S), formation and preservation of the soil structure (Lupwayi et al., 2011). Therefore, considering that soil microorganisms are strongly affected by crop management systems

(Aschi et al., 2017; Geisseler et al., 2017), it can be said that soil and plant management systems supporting the development of various microbial communities are the key to sustainable agriculture.

Soils under crop rotation systems with legumes usually have more microbial biomass (Moore et al., 2000; Adeboye et al., 2006) and bacterial diversity (Alvey et al., 2003; Qiang et al., 2004; Aschi et al., 2017) than soils under monoculture systems. In addition, the legume-cereal intercropping system is a system that provides better resource conversion efficiency, triggering higher biomass production while also improving the microbial activity of soils (Hailu and Geremu, 2021). Therefore, the cultivation of legumes both in crop rotation systems and in intercropping systems with other crops affects soil organisms and the many beneficial processes they mediate.

Yusuf et al. (2009) observed that the corn-cowpea crop rotation system increased soil MBC and MBN by approximately 30% and 200%, respectively, compared to the monoculture cultivation of corn in Nigeria.

It was revealed that lentils included instead of fallow in the fallow-wheat rotation system traditionally applied in the Central Anatolia Region of Turkey enriched the microbiological activity of the soil (i.e. β -Glucosidase and dehydrogenase activity) compared to fallow, and this activity was much higher, especially in the 0-20 cm fertility layer of the soil (Adak et al., 1998). Ramos et al. (2011) reported that legume cover in orchards improved the biological properties of soils by increasing enzyme activity. Qian et al. (2015) indicated that living

mulches consisting of *Coronilla varia* L. and *Trifolium repens* L. in apple orchards significantly improved soil bacterial activity, increased bacterial diversity, and changed the structure and function of the soil bacterial community. As stated by Zhong et al. (2018), mulching with *Chamaecrista rotundifolia* and *Arachis pintoii* legume forage crops in the subtropical and tropical persimmon orchards of China significantly changed the soil bacteria richness and diversity.

It was found that rice-wheat-mungbean rotation increased the arylsulfatase (41%), dehydrogenase (38%) and phosphatase (32%) enzyme activities of soils compared to rice-wheat rotation; rice-chickpea rotation had higher alkaline phosphatase (10%), β -glucosidase (13%) and protease (6%) activities than rice-wheat rotation. The same study also reported that high microbial activity in rotations containing legumes significantly affected the yield of the main crop (Borase et al., 2020). Some other researchers (Li et al., 2004; Ndakidemi, 2006) also stated that grain legumes secrete higher amounts of phosphatase enzyme than cereals.

It was revealed that urease and dehydrogenase enzyme activities in the post-harvest soils of corn and wheat were significantly higher in the forage faba bean+corn+wheat rotation compared to the corn-wheat rotation (Sürücü et al., 2014).

It was reported that the soils under the corn-wheat-corn-wheat-corn-wheat-alfalfa-alfalfa-alfalfa rotation system generally had higher hydrolytic enzyme activities than the corn-corn and corn-wheat systems (Giacometti et al., 2021).

It was identified that when *Vicia villosa* and *Vicia pannonica* were used as cover crops in apricot orchards (Demir et al., 2019a), *Trifolium repens* and *Vicia villosa* in kiwifruit (Demir and Isik, 2019) and hazelnut (Demir and Isik, 2020) orchards, the highest basal soil respiration values were obtained.

The Importance of Legumes for Sustainable Crop Productivity and Quality

To call a system sustainable, there must be a non-decreasing trend in yield. The stability of yield is an important feature that should be considered when evaluating the value of a cropping system in comparison with others (Berzsenyi et al., 2000). Crop rotation is a key component for the development of sustainable agricultural cropping systems (Wortman et al., 2011; Ceritoğlu and Erman, 2019).

Legumes, one of the richest sources of proteins, minerals, and fibers for animals and humans, play an important role in maintaining soil fertility, improving the physical environment of the soil, and increasing soil microbial activity (Gogoi et al., 2018). Due to these positive roles in improving soil health, legumes are an important component of both the intercropping system (Gogoi et al., 2018) and the crop rotation system.

Legumes have important and positive effects on the yield and quality of crops that come after them by being included in crop rotation systems and also increase agricultural efficiency and productivity by taking place in intercropping systems.

In the study conducted under the irrigated conditions of the Bursa region of Turkey, significant increases were obtained in the grain yield

of corn with the inclusion of legumes such as beans and peas in the crop rotation (Uzun et al., 2005). Again, a higher grain yield of sunflower was achieved from the Hungarian vetch-sunflower rotation application in comparison with the wheat-sunflower rotation system traditionally applied in the Thrace region of Turkey. It was reported that the crude protein content and the amount of wet gluten of wheat grains increased in the crop rotation systems, including Hungarian vetch and forage pea (Kaya, 2009). Additionally, Adak and Avcı Birsin (2000) stated that the protein ratio of wheat after lentils was higher than that of fallow-wheat and wheat-wheat systems.

Compared to the corn-corn rotation system, it was reported that the corn dry matter and grain yields were higher in legume-corn double rotation systems in which legumes such as velvet bean, cowpea, and soybean were cultivated before corn, the difference between legumes in terms of these yield increases in corn was also significant and the velvet bean-corn rotation system caused significant increases in corn yield over 100% compared to the corn-corn system (Uzoh et al., 2019).

Some other studies (Honeycutt et al., 1996; Berzsenyi et al., 2000; Li et al., 2009; Xuan et al., 2012) also emphasized that legumes in crop rotation systems contributed significantly to the yield of the following crops such as cereals and tuberous plants due to the nitrogen and other nutrients they added to the soil.

It was reported that forage biomass production increased by 52%, 13%, and 28%, respectively, in intercropping systems with sorghum, cowpea (70:30) (Zamir et al., 2016), lima bean (100:20) (Reza et al., 2012), and

soybean (Fidelis et al., 2016) in the same row. Again, different results were obtained in terms of forage yield in intercropping systems of sorghum and legumes with different row numbers. For example, while El-Sarag (2013) achieved a 9% increase in biomass by sowing sorghum and cowpea in 1-1 rows, Surve et al. (2012) ensured a 55% increase in biomass by cultivating the same plants in 2-1 rows.

Legumes in the intercropping system are also the insurance of agricultural production since they increase plant production per unit area. Thus, Hailu and Geremu (2021) reported that the intercropping of legumes with sorghum significantly increased the yield per unit area compared to the monoculture system, and Wang et al. (2021) stated that the sweet maize-soybean intercropping model could increase crop productivity.

Well-planned crop rotation, including legumes, not only improves soil properties but also reduces the mineral fertilizer requirements of subsequent crops. In other words, the inclusion of legumes in crop rotation systems is essential for the reduction of N fertilizer requirements for the production of many basic cultivated plants and for the sustainable management of agricultural systems.

In this sense, legumes in crop rotation reduce the fertilizer requirement of the crop and the next crop due to their residual effect and provide savings, especially in N applications (Bedoussac et al., 2015; Plaza-Bonilla et al., 2018; Hirzel et al., 2021).

It was reported that the winter-grown hairy vetch (*Vicia villosa* Roth.) could meet all of the N fertilizer required for the grain sorghum [*Sorghum bicolor* (L.) Moench] grown after it and up to 2/3 of the nitrogen necessary for the production of corn (*Z. mays* L.) (McVay et al., 1989).

It was indicated that including legumes in rotation during fallow periods between sugarcane cropping cycles could improve soil health, and the mineralization of both the nitrogen fixed in the soil by legumes during this period and the nitrogen in legume crop residues had the potential to reduce or eliminate the amount of the fertilizer N required for the sugarcane crop after legumes (Garside and Berthelsen, 2004).

It was stated that in rotational cropping of three leguminous species (*Mucuna pruriens*, *Canavalia ensiformis*, and soybean) with corn, *Mucuna* and *Canavalia* species provided significant organic matter to the soil, preserved soil moisture by covering the soil surface and the successive cropping of especially *Mucuna* with corn was the best system in terms of both soil organic matter and economic gain. The same study reported that corn planted after legumes could not meet all of the nitrogen needed for the optimum yield; however, a 60 kg N/ha application to corn planted after *Mucuna* was the most efficient management system, and this system provided approximately 30 kg/ha N savings compared to other legumes (Osei Bonsu and Asibuo, 2013).

In a study conducted by Özyazıcı et al. (2009) in the coastal part of the Central Black Sea Region of Turkey, it was determined that when forage faba bean (*Vicia faba* L.) was cultivated for green manuring in

the winter intermediate period within the scope of forage faba bean+corn+wheat rotation, applying 120 kg/ha N to corn and 100 kg/ha N to wheat grown after corn was sufficient for the optimum grain yield. Thus, it was reported that 40 kg/ha N in corn and 100 kg/ha N in wheat grown after corn were saved compared to the traditional system (corn-wheat). The same study emphasized that the grass of forage faba bean could also be used as animal feed by ignoring the use of 20 kg/ha more N for corn in comparison with the traditional system and saving 50 kg/ha N for wheat. In the study, it was also determined that the crude protein contents of corn and wheat grains were higher than the control application in both cases when forage faba bean was cultivated for both green manuring and grass.

Significant increases in the yield and quality of crops can be achieved in cropping systems in which legumes are used as cover crops. It was found that fruit yields were significantly higher in applications when *Vicia villosa* and *Vicia pannonica* were used as cover crops in apricot orchards (Demir et al., 2019a), *Trifolium repens* L., *Vicia villosa* Roth. and *Trifolium meneghinianum* Celm were used as cover crops in persimmon orchards (Demir and Işık, 2019a), *Vicia villosa* and *Trifolium meneghinianum* in kiwifruit orchards (Demir and Isik, 2019), *Vicia villosa* in hazelnut (Demir and Isik, 2020) and persimmon (Macit et al., 2020) orchards compared to other alternative cover crop applications. Furthermore, it was reported that fruit yields improved by cultivating some legume forage crops as intermediate or cover crops in orchards (Srivastava et al., 2007).

CONCLUSION

Crop rotation must be an alternative strategy to preserve soil and plant productivity. Additionally, including cover crops in crop rotation can further improve soil organic matter, nutrients and moisture holding capacity and reduce water and soil loss. The intercropping system is the economic insurance of plant production. The sustainability of all these systems both in terms of the physico-chemical and biochemical quality of the soil and in terms of plant production is only possible with the presence of legumes. Agricultural systems, including legumes, are the insurance of sustainable agriculture by increasing soil vitality and soil fertility without reducing the yield of crops coming after them in the long term.

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CHAPTER 2

GRAIN LEGUMES UNDER ABIOTIC STRESS

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INTRODUCTION

Global climate change, the impact of which we are starting to feel intensely today, reminds us once again of the importance of providing sufficient and quality food to the increasing world population. The effect of global warming is seen differently in different geographies of the world. This effect manifests itself as warming in some regions, cooling in some regions, and an increase-decrease in precipitation in some regions. It should not be forgotten that the climate changes brought about by global warming will vary positively and negatively according to regions in the world (Şen, 2014).

If the increasing rate of CO₂ in the atmosphere continues in this way, an increase of 3° C in the average temperature of the world is expected in the near future due to the greenhouse effect. The world population is expected to reach 9 billion (Türkan et al., 2015). According to the Food and Agriculture Organization (FAO), the population is increasing rapidly and will reach approximately 10 billion in 2050. Moreover, food production is decreasing because of various abiotic stresses. In this context, the effect of biotic and abiotic stress factors in plant cultivation will be felt even more. High temperature and drought are perhaps the most stressed conditions for plants. Excessive salt accumulation in the soil and aluminum toxicity, which is the characteristic problem of acidic soils, are other important stress factors that limit plant production (Çakmak, 2015). In addition to these, cold stress can cause various growth disorders and yield reduction in plants. Incorrect or excessive use of pesticides can also be counted among the abiotic stress factors

that cause various problems in plants. Abiotic stress factors have significant effects on agricultural production systems at critical stages of plant growth, such as seed germination and early seedling growth, and adversely affect yield. To solve this problem, chemical and physical treatments applied to seeds before sowing is rapidly developing as a promising agricultural stress management technique that increases germination rate and provides simultaneous emergence under salinity and drought conditions. Magnetic field application, which is one of the physical methods applied to seeds, has come to the fore in recent years as an application that increases the tolerance of agricultural products against stressful environmental conditions. The applied magnetic field interacts with the seeds, accelerating the metabolism, ultimately improving germination, activation of primary and secondary metabolites, enzyme activities, nutrient and water uptake. As a result, magnetic field applications have a positive effect on plants not only in the early stages of plant growth and development but throughout the entire development process (Sevilmiş et al. 2020).

Grain legumes, which have a very important place in a balanced and quality diet, consist of dry beans, peas, cowpeas, beans, lentils and chickpeas. One of the basic elements of a balanced diet is proteins, and it must be met from plant and animal foods in daily diets. Grain legumes are very important in closing the vegetable protein deficit. In addition to Asian and African countries, there has been an increase in demand for legumes in the United States and some wealthy Western European countries. It is stated that the reason for this is that legumes contain high

levels of the essential amino acid lysine (lysine) and some nutritional properties such as low cholesterol levels begin to emerge more clearly. 22% of vegetable proteins and 7% of carbohydrates in human nutrition in the world; 38% of protein and 5% of carbohydrates in animal nutrition are provided from legumes. Thus, grain legumes, which contain 18–31.6% protein in their composition, constitute a more efficient and economical plant group in solving the nutritional problem of countries and eliminating the protein deficit in nutrition. In other words, legumes are the protein source of 2 billion people in the world (Adak et al. 2010). They are indispensable sources not only for human nutrition but also for the supply of animal feeds with high protein content. While they meet their own nitrogen needs due to their nitrogen fixation ability to free nitrogen from the air, they are also indispensable plants of the crop rotation, as the remaining wastes in the soil create a nitrogen-rich environment. This is very important for the understanding of sustainable soil and the environment. It reduces the need for nitrogen fertilizer use as it creates a nitrogen-rich environment for the plant to be planted after it is used very little or not at all in the lands where it is cultivated. Denitrification, which may occur in airless environments as a result of too much or incorrect use of nitrogen fertilizer, contributes to global warming is quite high. It is a fact that during the global climate change process, the increase in carbon dioxide and temperature in the atmosphere will increase drought and salinity. It is thought that these changes will adversely affect legumes grown in arid and semi-arid climates. In this sense, it is important to study the effects of these negativities on the root-nodule formation and biological nitrogen

fixation. It has been reported that the increase in the symbiotic relationship between legumes and bacteria with increasing CO₂ conditions accelerated nodule development and increased biological nitrogen fixation. On the other hand, according to climate scenarios, it has been reported that 2-4 °C temperature increase and drought together with it have been found to adversely affect biological nitrogen fixation by reducing root hair infection, number of nodules, nodule size, nodule development and activity. On the other hand, it has been emphasized that salinity reduces nodule respiration and leghemoglobin content (Yavaş and Ünay, 2018). For such reasons, it is necessary to increase the legume cultivation areas, to eliminate the stress conditions that may be exposed in cultivation, or to reduce their effects. For this purpose, studies on legume cultivation under various abiotic stress conditions in recent years have been researched and tried to recover. In this study, abiotic stress conditions that grain legumes are exposed to are discussed and explained separately and summaries of some recent studies on each stress condition are presented.

DROUGHT (WATER) STRESS

Drought refers to the period in which there is not enough rainfall until the amount of moisture in the soil drops to the amount where the plants suffer from lack of water. Drought is an important factor limiting crop production in most of the world's agricultural lands. Among the stress factors seen in arable lands around the world, drought stress takes the biggest slice with a share of 26%. Drought stress causes many physiological, biochemical and molecular events in plants, and

accordingly, plants can develop tolerance mechanisms that will enable them to adapt to limited environmental conditions (Özcan, 2020).

Drought begins as a meteorological drought, develops as an agricultural and hydrological drought, and its effects become visible as a socio-economic drought. While meteorological drought is based on precipitation and is defined as the precipitation below normal values for a long time, agricultural drought is defined as the lack of water in the soil to meet the needs of the plant (Mengü et al., 2011). While hydrological drought is the decreasing amount of water in rivers, lakes and underground water resources, socio-economic drought includes the deterioration of economic balances due to other drought types (Mishra and Singh, 2010).

Responses to drought stress can occur through morphological, physiological and biochemical pathways (Anjum et al., 2011). One of the early effects of water deficiency is a decrease in vegetative growth due to a decrease in the rate of photosynthesis. Stem growth and especially leaf growth are more sensitive to water deficiency than root growth (Sağlam, 2004). In the first periods of dry conditions, the plant slows down stem elongation and triggers root development in order to reach more water (Öztürk, 2015). While protein and chlorophyll synthesis in plants are negatively affected in drought, seeds lose their germination ability and are negatively affected in photosynthesis and respiration (Kacar et al., 2006). Many researchers have stated that drought stress negatively affects photosynthesis (Ruiz-Lozano et al., 2012; Osakabe et al., 2014, Razzaghi et al., 2014). It is known that

drought stress has significant effects on enzyme activity and enzyme amounts in plants. While the amount of ABA increases 40 times in the leaves, this increase is less in other organs including the root. ABA prevents stomata from closing and water loss by transpiration. It reduces the development of the top organs of the plant and causes the use of water in the root system and thus the root elongation (Kacar et al., 2006).

Various studies have been carried out at physiological, molecular and biochemical levels to understand the effects of drought stress on various plants. Some of these studies on edible legumes were compiled in this study. Drought is the abiotic factor that most adversely affects legume production. It turns out, however, that the largest producers of pulses (70% of global production) are located in regions that experience water shortage (e.g., India, China and many African countries) (Gowda et al., 2009) Since the effects of drought on legume production were found to be less affected by climatic regions (e.g., non-tropical vs. tropics or drylands vs. non-drylands) but was more related to legume species, the selection and promotion of drought-resistant legume species could provide an approach to minimize the impact of droughts. For legumes, drought stress has adverse effects on total biomass, pod number, seed number, seed weight and quality, and seed yield per plant (Toker et al., 2007; Charlson et al., 2009; Khan et al., 2010; Toker and Mutlu, 2011; Impa et al., 2012; Hasanuzzaman et al., 2013; Pagano, 2014). Faba bean and pea are known to be drought-sensitive, whereas lentil and chickpea are known as drought-resistant genera (Toker and Yadav, 2010).

Lentils (*Lens culinaris* Medik.) is one of the legume plants affected by drought stress and lost 6-54% yield (Oweis et al., 2004; Stoddard et al., 2006; Sehgal et al., 2017). By breeding different genotypes of lentil lines, specific breeding programs have been initiated to reach drought-tolerant varieties. (Singh et al., 2016). Recently, genome-wide studies have been reported on leaf tissues of drought-sensitive and resistant lentil lines to identify the affected biological process (Singh et al, 2017). Bakır (2019) isolated the partial cDNA of the lentil (*Lens culinaris* M.) DREB2A gene, which is a member of the DREB gene family and named LcDREB2A, and determined its relationship with drought stress. They found that LcDREB2A gene expression increased with increasing drought stress in the leaves of Fırat87 and Özbek cultivars and reached the highest level on the 20th day of drought, while the gene expression level in the roots of the Özbek cultivar reached the highest level on the 6th day, decreased on the 13th day and increased again on the 20th day. reported. They stated that in the Fırat-87 variety, a regular increase was observed with increasing drought stress and reached the highest level on the 20th day. Morgil (2019) subjected the samples to de novo RNA sequencing-based transcriptome analysis after establishing drought stress conditions for lentils to learn about the transcriptional rearrangements occurring throughout the genome in the roots and leaves of lentil seedlings grown under short- and long-term drought conditions. Differentially expressed gene (DEG) analysis on these transcripts showed that drought stress duration had a greater effect on transcriptional regulation in lentil root. Also, gene ontology analysis; revealed that biological processes such as protein phosphorylation,

signal transduction, regulation of transcription, DNA replication and preservation of root meristem identity are differentially regulated in response to prolonged drought stress. Drought tolerance mechanisms of 11 chickpeas (Menemen-92, Akçin, Aydin-92, İzmir-92, Kusmen, Canitez-87, Gokçe, Sarı, Uzunlu-99, Er-99 ve ILC-195) and 6 lentils (Malazgirt 89, Özbek, Fırat 87, Sazak 91, Emre 20 and Kayı 91) widely grown in Turkey were investigated in the study on the determination of drought-related oxidative stress and physiological tolerance mechanisms in lentil and chickpea plants. Drought stability indices (KHI) of cultivars grown in wet and dry conditions were determined. Responses or tolerance mechanisms of plants to drought (oxidative stress) with KHI are explained by the formation of H₂O₂, lipid peroxidation and damage to their membranes and accumulation of proline and ascorbic acid. In addition, physiological parameters such as stomata resistance, plant temperature, relative chlorophyll, relative moisture content, leaf water holding capacity, which are used or have the potential to be used in the selection of drought-tolerant varieties, were determined. The relationships between these physiological parameters and the biochemical changes in the plant and the changes in the mineral nutrition performance of the drought on chickpea varieties were also revealed. In addition, the nutritional (N, P, K, Ca, Mg, Fe, Zn, Mn and B) performances of drought-resistant cultivars were determined and significant relationships were determined between nutrient utilization efficiency and drought tolerance. Researchers divided chickpea and lentil varieties into two groups according to their KHI as drought-sensitive and resistant. Accordingly, Akcin, Aydin-92, Er-99,

Uzunlu-99 and Menemen-92 chickpea cultivars and Fırat 87, Sazak 91 and Emre 20 lentil cultivars were accepted as drought-resistant varieties compared to other cultivars. In general, ascorbic acid content in chickpea cultivars increases with drought, proline accumulation occurs under drought stress in chickpea and lentil cultivars, important relationships were determined between KHI and proline accumulation, proline levels are an important parameter in determining drought tolerance, and H₂O₂ concentration, lipid peroxidation and lipid peroxidation of plants. reported an increase in membrane permeability. (Güneş et al., 2006).

Katerji et al. (2001) in the lysimeter study in which they investigated the responses of 2 chickpea cultivars with different drought tolerance to soil salinity, drought-resistant and sensitive chickpea cultivars yielded the same product under salt-free conditions; In saline conditions, there was an early senescence effect on the leaf and dry matter development and flowering of the drought-resistant chickpea variety; On the other hand, the drought-sensitive chickpea species showed a different behavior due to the development of new leaves and flowers on the 135th day after planting in slightly salty conditions (EC: 2.5 dS/m), the senescence effect was delayed and the same product was obtained as chickpeas grown in salt-free conditions; In salty conditions, it was determined that a product reduction of 70% occurred in both chickpea species. In another study on chickpea, they evaluated 482 chickpea cultivars in terms of drought resistance and determined 18 tolerant cultivars according to the drought response index. They stated that these

resistant species yield higher than 10% yield in dry conditions, dry root weight, root volume and rooting depth decrease significantly and leaf area decrease in sensitive varieties at low moisture levels (Anbessa and Bejiga 2002). Günes et al. (2005), in their study investigating the possibilities of using some physiological parameters as drought tolerance criteria in chickpea cultivars, created drought stress in 11 different chickpea cultivars before and after flowering in greenhouse conditions. It was determined that the responses of the varieties to the arid conditions created in both periods were different. They reported that the leaf relative humidity, ascorbic acid and proline contents of the drought-resistant cultivars were high, the leaf water holding capacity and membrane permeability was low.

Gürbüz et al. (2009) investigated the effects of drought stress induced by PEG 6000 on the germination properties of grain size and drought stress in some chickpea (Dikbaş, Gökçe and Uzunlu-99) cultivars. In this study, they determined the effects of grains of different sizes (7, 8 and 9 mm) on germination. They reported that there was a difference between varieties and grain size in terms of germination characteristics under drought stress, Gökçe variety germinated at a high rate in arid conditions, while small (7 mm) grained varieties gave better results than medium (8 mm) and coarse (9 mm) grains. They stated that the germination rates of all varieties in all grain sizes decreased under -4 atm drought stress, and if chickpea cultivation is to be carried out in arid conditions, besides the drought-tolerant chickpea varieties, the small grains of these varieties will provide fast and uniform

germination. Çevik and Ünyayar (2015) used cultured chickpeas *Cicer arietinum* ILC482 (drought-sensitive) and wild chickpea *Cicer reticulatum* AWC611 (drought resistant) with different drought resistance in their study investigating the effect of drought stress on protein expression in two chickpea species with different tolerances. They stated that drought stress did not change the stem length in both species but decreased the root length, and this decrease was more in *C. arietinum*. Drought stress decreased the leaf water potential and leaf proportional water content in both species, these decreases were much more pronounced in *C. arietinum*, besides, the amount of proline increased in both species with drought stress, but in *C. reticulatum*, the results of drought stress. They found that the increase in the amount of proline was four times higher than that of *C. arietinum*. The expression of 11 proteins in *C. arietinum* changes with drought stress, while the expression of six of these proteins increases with drought stress, while the expression of five decreases. In *C. reticulatum*, on the other hand, they reported that the expression of 13 proteins changed with drought stress, while the expression of 11 of these proteins increased under drought stress, while the expression of two decreased. When they examined the proteins whose expression changed, they found that most of these proteins were proteins involved in the photosynthesis mechanism and when the expression changes in both species were interpreted, both species responded in different ways in the complex stress response. They reported that in *C. arietinum* there was a response resulting from the signals created by more active oxygen species, while a proteome response occurred in *C. reticulatum* upon the preservation

of cellular water. Yürekli (2015) investigated the effects of abscisic acid (ABA) and nitric oxide (NO) in chickpea plants under drought stress. In the study, NO level, proline amount, lipoxygenase activity, malondialdehyde (MDA) level, total chlorophyll, total protein amount and proportional water content (OSI) were investigated. As a result of the study, it was determined that there were increases in all parameters in susceptible chickpea cultivars compared to resistant chickpea cultivars except OSI amounts. It was also determined that in drought-sensitive cultivars, stress-protective molecules such as ABA, NO and proline, which play an important role in the stress response, increased more.

Franca et al. (2000), in their study aiming to determine the effects of drought on the development and plant-water relations of bean varieties in greenhouse conditions, 4 bean varieties (A320, Carioca, Ouro Negro and Xodo) were grown under water stress and their adaptation mechanisms were growth parameters, water status, gas exchange (stomatal resistance). The growth rate showed significant differences between the cultivars depending on the drought. They reported that at low water stress ($w = -0.60$ MPa) the stomata of the A320 variety were completely closed, whereas the other varieties were closed under the stress condition of $w = -0.90$ MPa and the net assimilation rate was completely related to the stomatal opening. Although Carioca and Ouro Negro cultivars have high water holding capacity and better tolerance mechanism in water stress than the other two cultivars in terms of yield, the resistant cultivars (Carioca and Ouro Negro) have high leaf osmotic

potentials and membrane stability and as a result, they are grown in the same ecological conditions. They stated that different bean cultivars have significantly different tolerance mechanisms in terms of drought tolerance. Boutraa and Sanders (2001) investigated the effects of water stress on yield and yield components of bean cultivars (Carioca and Prince) under greenhouse conditions. Plants were grown in optimum conditions, under water stress during flowering and fruit (pod) setting. Researchers reported that Carioca cultivar is more drought-resistant than Prince, and water stress applied in both periods negatively affects the development and yield elements of plants (grain weight, number of grains per plant, number of pods per plant, number of leaves, plant height). Kırıcı (2019) investigated the expression levels of YABBY and DOF genes in 2 different beans (*Phaseolus vulgaris* L.) cultivars (Yakutiye-98, Zülbiye) under drought stress conditions. The effects of salicylic acid application on plants under drought stress were examined at the level of mRNA expression for these genes. Relative water content (RWC) and morphological changes were compared by applying salicylic acid to plant samples under drought stress, and the changes observed in the expression levels of transcription factors, the effects of stress and hormone application were investigated. In their study to determine the effect of drought stress on plant growth, nutrient content, and some physiological and biochemical properties in beans, they reported that drought stress had a negative effect on leaf area, leaf-stem-root wet weight and leaf-stem-root dry weight in beans. At 60% irrigation level, leaf fresh weight, stem wet weight, root fresh weight, leaf dry weight, stem dry weight and root dry weight decreased by 17,

33, 55, 57, 60 and 52 %, respectively, compared to 100% irrigation level. They reported that while (EC) increased with drought stress, tissue proportional water content (DSI) decreased. In the study, peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activity in the leaves increased at 80% irrigation level, but decreased significantly at 60% level, while drought stress also decreased the plant nutrient content in the examined organs in beans, on the other hand, hydrogen peroxide was observed. (H₂O₂), malondialdehyde (MDA), proline and sucrose content were reported to increase (Yıldırım et al. 2020). Kabay and Şensoy (2021), in order to determine the enzyme and ion exchanges caused by drought in bean plants in drought stress-tolerant (Yakutiye and V-a1) and sensitive (Zulbiye and T7) bean genotypes, enzymes and ions (catalase (CAT), examined the changes in superoxide dismutase (SOD), ascorbate peroxidase (APX), malondialdehyde (MDA), Chlorophyll-a, Chlorophyll-b, total chlorophyll, K, Ca and Na and reported that there were significant differences between them. Amorim et al (2018) conducted a study to identify genes and pathways that confer stress tolerance, due to the ability of cowpea to fix nitrogen in poor soils and its relative tolerance to drought and salt stresses. In the study, four different cowpea genotypes were rigorously tested for traits of nine candidate reference genes under two abiotic stress conditions. They reported that there are suitable reference genes for qPCR analyzes in cowpea under root dehydration and salt stress.

Low soil moisture in faba bean significantly reduced the number of infection filaments. Deformation of outer cortical cells occurred in faba bean plants exposed to drought. Drought also affected symbiotic nitrogen fixation by causing lower nitrogenase activity. This is also important for legumes, resulting in lower nitrogen for protein biosynthesis and reduced grain yield (Farooq et al., 2016). During the pod-filling phase, the sensitivity of peas to water stress is lower reducing mainly the number of pod seeds (Mahieu et al., 2009).

Magnetopriming (exposure of seeds to a magnetic field) shows a physical stimulant property that improves the seed strength under abiotic stress and helps the plant stand to form properly (Anand, 2014). In abiotic stress conditions such as drought and salt in the soil, magnetopriming reduces the negative effects of stress by increasing antioxidants in plants and reducing oxidative stress (Radhakrishnan, 2019). In another study, desi and kabuli type chickpea seeds were treated with a static magnetic field before sowing and it was observed that light utilization efficiency and water usage efficiency increased under water stress. Seeds exposed to treatment (100 mT, 1 hour) increased root volume and surface area by 70% and 65%, respectively. The application allowed the plants to absorb 60% higher moisture during the active growth period (78-118 days after planting) without loss of soil moisture. Both applied genotypes had better water use efficiency, biomass and radiation utilization efficiency (Mridha et al., 2016).

SALT STRESS

Salinity is among the most important problems threatening arid and semi-arid areas. The increase in salinity in agricultural areas deteriorates the structure of the soil and significantly limits the product quality and productivity of plants. Salt stress causes many disruptions at the morphological, cellular, physiological and molecular levels in addition to various developmental processes in plants. Plants develop various tolerance strategies in response to salt stress. Salt stress, in parallel with the increase in the amount of NaCl and other soluble salts in the soil, causes negative effects on the growth and development of the plant. Increasing the salt concentration in the soil solution and decreasing the water potential decrease the osmotic potential of the plant cells and cause a series of reactions in the plants. Therefore, salt stress affects many biological events such as growth, development, germination, cell division and photosynthesis in plants depending on its intensity and duration, and salinity limits plant productivity and product quality in agricultural areas (Özcan, 2020). As with many stress factors, plants can show different levels of response/tolerance to salt stress during germination, early seedling and mature plant periods. Leguminous plants are generally known to be sensitive to salt stress (Tiryaki, 2018; Ceritoglu et al., 2020). Salt stress causes significant reductions in the number of nodules and the amount of fixed nitrogen in legumes. Salt negatively affects rhizobial colony formation and early bacterial infection processes, especially in roots (Zahran, 2001). N fixation in legumes exposed to salt stress during developmental stages

shows great sensitivity to salt stress (Boukhatem et al., 2012). Katerji et al. (2001) stated that salinity affects seedling survival, germination, leaf water potential, maximum osmotic balance, leaf area development, dry matter, number of flowers and yield in 2 lentil cultivars. While it is reported that the efficiency loss is 2% when /m, the efficiency loss is 90-100% when 3dS/m. Steppuhn et al (2001) studied the effects of salinity on these plants using different salt doses (1.2, 11.2 and 24.9 dS/m) and bean, pea and wheat plants. They reported that increasing salt concentrations decreased the germination rate in all plants, plant death occurred in peas in the first development period, biomass weight decreased and yield decreased by 40%, among these plants, wheat was more tolerant to salinity than peas and beans. Silva et al. (2003) found that the soluble carbohydrate amounts of different NaCl in cowpea (*Vigna unguiculata*) increased with increasing NaCl concentration. Researchers examining the effects of different NaCl concentrations on germination and seedling growth of 95 dry bean (*Phaseolus vulgaris* L.) genotypes reported that germination and seedling growth decreased depending on the increase in salinity, but there was variation in salt tolerance among 95 genotypes tested for germination and emergence (Elkoca et al., 2003). The effects of salicylic acid (SA) applied to black chickpea (*Cicer arietinum* L.) seeds after germination on germination and initial growth under saline conditions were investigated, and it was observed that increasing salt doses inhibited the germination and growth parameters of black chickpea (*Cicer arietinum* L.) seeds. Contrary to salt doses, it was determined that increasing salicylic acid (SA) doses had a positive and significant effect on germination and

growth, and gradual increases in salicylic acid doses in parallel with increasing salt doses reduced the suppressive and harmful effects of salt (Baran et al., 2019; Ceritoglu and Erman, 2020).

Among the local populations commonly produced by producers in Kastamonu region, flower okra (*Abelmoschus esculentus* L.), pepper (*Capsicum annuum* L.), chickpea (*Cicer arietinum* L.), cucumber (*Cucumis sativus* L.), walnut (*Juglans regia* L.), lentil (*Lens culinaris* L.), pea (*Pisum sativum* L.), sugar bean (*Phaseolus vulgaris* L.), eggplant (*Solanum melongena* L.), spinach (*Spinaceae oleraceae* L.), einkorn (*Triticum monococcum* L.), gum broad bean (*Vicia faba* L.) and sweet corn (*Zea mays* var. *saccharata*) were applied in 13 different types of salt, 75, 150 and 225 mM. Photosynthetic pigments, soluble protein, proline, lipid peroxidation (MAD-H₂O₂) amounts were measured as durability criteria. The effects of salt applications on photosynthetic pigments in einkorn, eggplant, cucumber and okra; proline in broad beans, peas, okra, walnuts, spinach, chickpeas, eggplant, cucumber and einkorn; It increased the protein content of peas, peppers, walnuts, chickpeas and eggplant, and decreased the MDA content of chickpeas, spinach, sugar, broad beans and einkorn. The most resistant to salt were einkorn, eggplant, chickpea, walnut and cucumber. In addition, the effect of salt stress differed depending on the plant species and salt concentrations (Turfan, 2015).

Çekiç and Yılmaz (2015) conducted a study to determine the content of gamma aminobutyric acid (GABA), one of the stress metabolites, in arid and saline stress conditions in two bean varieties. PEG application

did not affect significantly GABA content of *P. vulgaris* cv.Akman 98, whereas GABA content was increased by drought stress in *P. vulgaris* cv.Göynük 98. In the combined stress conditions, GABA content was decreased in *P. vulgaris* cv.Akman 98, whereas it was increased in *P. vulgaris* cv.Göynük 98. According to our findings, we can conclude that single stress conditions could enhance GABA content and GABA is strongly related to stress factors. However, in the combined stress conditions plants could give different responses. Further analyses should be done to evaluate the effects of multiple stress conditions in different plants.

The effect of salt stress on the germination of chickpea seeds, how it affects the regulation of reactive oxygen species (ROS) in seed embryos under stress and the activities of some antioxidant enzymes responsible for ROS scavenging, how ROS regulation and antioxidant defense system change with the application of brassinosteroids (BRs) to seed embryos exposed to salt stress. The relationship between BRs and ROS regulation during radicular elongation and development was investigated. Seed germination efficiency was determined depending on the increased amount of ROS. Salt stress (200 mM NaCl) caused inhibition of seed germination, but improved germination of seeds exposed to stress with external application of BRs. On the other hand, changes in antioxidant defense systems such as superoxide dismutase (SOD), NADP-H oxidase (NOX), peroxidase (POX) and catalase (CAT) were determined by associating BRs application, which has a known role in the recovery of seed germination under stress, and ROS

amounts under stress. The role of the antioxidant system in germination was clarified by promoting ROS production. The study of Dadaşoğlu et al. (2020) investigated the effects of 4 different salt concentrations (50, 100, 150 and 200 mM NaCl) on seed germination in 4 chickpeas (Canada, İnci, Azkan and Çağatay) and 4 peas (Utrillo, Serge, Jof and Bolero) cultivars. It was reported that with increasing salt levels, significant decreases in germination properties were observed in both species. They reported that while pea cultivars did not germinate at 150 mM (except Jof) and 200 mM salt stress, chickpea cultivars did not germinate at 200 mM (except Çağatay) salt stress, and the critical limit salt level for germination should be considered as 100 mM for both species. Another study was conducted to determine the effect of 7 different salt concentrations (0, 25, 50, 75, 100, 125, 150 mM NaCl) on plant growth in pea genotypes. To determine the salinity tolerance of pea genotypes, plant height, root length, number of leaves, SPAD value, above-ground fresh weight, above-ground dry weight, root fresh and root dry weights and Na concentration of above-ground parts were investigated. It has been determined that there are significant differences between genotypes in terms of their responses to the applied salt doses, and Na accumulates in the above-ground parts from 25 mM salt dose in all genotypes (Zambi, 2019). The amounts of malondialdehyde, hydrogen peroxide, total phenolic compounds, anthocyanins and sinapoyl esters in plant leaves were also investigated by applying 50 mM and 100 mM NaCl to pea varieties (Sprinter and Utrillo) for 10 days. Salt stress partially decreased the amount of malondialdehyde in both cultivars. While the amount of hydrogen

peroxide increased significantly in Sprinter depending on the increasing salt amount, it increased partially in Utrillo. While salt stress significantly increased the total amount of phenolic substances in pea varieties depending on the concentration, it did not affect the amount of anthocyanin and sinapoyl ester (Tetiktabanlar et al., 2020).

Kızıll Şimşek (2013) stated that salty soils negatively affect bean cultivation, and compared the expression of the DREB2A gene, whose activation is increased in the application of salt stress in organic and conventional bean (*Phaseolus vulgaris* L.) cultivation. She reported that amplification was observed in melting curve analyzes for bean actin and DREB2A. Fidan and Ekinialp (2017) investigated their responses to salt stress by applying 25 mM and 50 mM salt (NaCl) doses to 20 different bean genotypes. They applied salt with irrigation water for 4 consecutive days and examined shoot-root length, number of leaves, shoot diameter, shoot fresh and dry weights and nutrient content (Na, Ca and K) in shoots. According to the results obtained, there are significant differences between genotypes in terms of salt tolerance, shoot length, number of leaves, shoot diameter, shoot fresh weight, K, K/Na and Ca/Na ratios decreased in parallel with the increase in applied salt concentration; They reported that the amount of Ca and Na increased. In a similar study, they investigated the effects of salt stress on the content of some nutrients (P, Mg, Fe, Cu, Mn and Zn) and total antioxidant and total phenol content in different bean genotypes in 20 bean genotypes collected from the Van Lake Basin. Salt was applied in three ways: 0 mM, 25 mM and 50 mM NaCl concentration. When the

rate of change of the plants under salt stress compared to the control was examined, it was reported that the total phenol content and antioxidant amount decreased at a high rate with the increase in the salt dose, and salt stress had a negative effect on the genotypes. Researchers found that P content increased in both salt doses and Fe content increased in 50 mM salt in green parts; It was determined that Mg, Cu and Zn contents were decreased at 25 mM and 50 mM salt doses, and Mn content was decreased in 50 mM NaCl application. They reported that the Fe, Mg and Mn contents of the root decreased at 25 mM and the P, Mg, Cu, Mn and Zn contents increased as the salt dose increased (Kipçak et al., 2019). The study carried out to determine the effects of different salt concentrations (0, 25, 50, 75, 100, 125, 150, 175, 200 mM) on plant growth in cowpea cultivars, plant height, root length, leaflet number, leaf area, fresh weight above the soil, dry weight above the soil, root wet weight, root dry weight and mineral substance concentration (Na, Ca and P) of above-ground parts were investigated. The interaction of cultivar x salt dose was found to be statistically significant in terms of plant height, root fresh weight, root dry weight and the amount of Na, Ca and P in the above soil green parts. Both cowpea cultivars were generally adversely affected by the increased salt dose (Altun, 2019).

COLD STRESS

Some plants are sensitive to low temperatures and suffer from dehydration under cold stress. In addition, cold stress led to oxidative stress, higher protoplasmic viscosity and catabolism and decreased rate

of photosynthesis in sensitive plants. Some plants, however, are tolerant to low temperatures and could acclimate to cold if kept in the low but non-freezing temperature for appropriate time. During cold acclimation, some metabolic changes such as activation of antioxidant enzymes, accumulation of soluble sugars, abscisic acid and proline, cessation of growth and formation of rosette stem, water loss and changes in membrane composition may occur (Doğru, 2019). High and low temperatures constitute the most damaging type of abiotic stress and limit the survival and productivity of plants. The present study aimed to evaluate the role of exogenous applications of acetylsalicylic acid (ASA) in reducing the deleterious effects of cold stress. *Phaseolus vulgaris* L. seedlings were treated with foliar-sprayed ASA at concentrations of 0–3 mM and then subjected to chilling stress at 4 °C for 2 or 4 days. Growth, photosynthesis, biochemical alterations, oxidative damage and antioxidant enzyme activities as well as the expression of cold-responsive genes (CBF3–COR47), were monitored during the experiment. ASA applications substantially improved some growth and photosynthetic parameters, including shoot biomass, dry weight, and photosynthetic pigments, of *P. vulgaris* seedlings exposed to different durations of chilling stresses. The ASA foliar spray treatments significantly rescued the growth and photosynthetic pigments of *P. vulgaris* seedlings under different chilling stresses. The total soluble sugars markedly increased during 0-4 days of chilling stress following ASA foliar spraying. The exogenous application of ASA significantly increased the accumulation of proline in *P. vulgaris* seedlings under chilling stress. At the gene expression level,

ASA significantly) upregulated the cold-responsive genes CBF3 and COR47 (Soliman et.al.2018)

Cold acclimation in plants is an effective process for improving cold tolerance and determining winter survival capacity. Evaluation of survival percentage and regrowth traits of plants after freezing stress is a very important criterion in cold-related studies. Hasanfard et.al. (2018) was carried out two faba bean landraces (Borujerd and Neyshabur) and 7 freezing temperatures (0, -4, -8, -12, -16, -20 and -24°C). Plants were cold acclimated in the natural environment till to 4-6 leaf stage before freezing stress was imposed in a thermogradient freezer. Results indicated that freezing temperatures significantly affected survival percentage and other regrowth traits in a way that all the traits were decreased as freezing temperature decreased. This was more severe in temperatures lower than -12°C. All the plants were killed at -16°C.

Didani et.al. (2015) applied some biochemical substances (progesterone, beta estradiol, aba, aspirin) and physical energies (electric field and ultraviolet light) in combination to increase cold resistance in beans. In addition, by investigating some biochemical parameters (protein amount, hydrogen peroxide (H₂O₂) amount, lipid peroxidation level) in beans applied to provide resistance to chilling, they tried to determine how these applications affect these parameters in a cold-sensitive plant. 10⁻⁶M β-estradiol, 10⁻⁹ M progesterone, 10⁻¹mM SA were applied to the plants that reached a certain size under controlled conditions by spraying the leaves. In addition, a 100 kV/m

50Hz DC electrical field created between two aluminum plates facing each other parallel to each other was applied to the seedlings for 10 minutes. UV application was made for 10 seconds. Then, the plants transferred to the climate cabinet after 4-5 hours (temperature 9/5 °C, humidity 70-60%, day length 14/10 hours' light/dark) were harvested after 3 days. They reported that protein content increased in all cold-applied plants, there was a noticeable increase and change in MDA level of lipid peroxidation (LPO) activity, and significant decreases in H₂O₂ amounts in the groups in which all physical energy and biochemical substances were applied. In another study, antioxidant system parameters of bean (*Phaseolus vulgaris* L. cv. Elkoca-05) treated with psychrophilic bacteria isolated from plant leaf apoplast were evaluated and its response to cold stress was investigated. Lipid peroxidation (MDA) and hydrogen peroxide (H₂O₂) and superoxide anion (O²⁻) levels from ROS compounds were determined in the leaves of the plants harvested 3 days after the cold transfer. In addition, catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) activities were measured in the same leaves. They reported that when the application of the three bacterial isolates used in the study compared with the controls, the antioxidant enzyme activities in beans generally increased, and cold alone increased the levels of H₂O₂, O₂⁻ and MDA. They reported that psychrophilic bacteria inoculated before exposure to cold stress contribute to increase tolerance to cold stress by reducing ROS levels and increasing the activity of antioxidant enzymes (Tiryaki and Atici, 2015).

In the seeds of chickpea plants cold stressed during seed development, Nayyar et al. (2005) reported increase in accumulation of sugars but decrease in accumulation of storage proteins, starch and several amino acids in chickpea seeds. Cold stress during the development of chickpea seeds has detrimental effects on seed yield in both Desi and Kabuli chickpea genotypes (Nayyar et al. 2007). Sucrose content decreased significantly in the leaves of cold-stressed plants. The seed growth rate, seed fill duration, seed number, and average seed weight and size decreased greatly in the plants cold-stressed at the late pod-filling stage than those stressed at the early pod-filling stage. A greater reduction was observed in starch, proteins, soluble sugars, fat, crude fibre and storage protein fractions in the seeds of the plants cold-stressed at the late pod-filling stage (Kaur et. al., 2008).

Under field conditions, growth parameters like fresh weight and leaf number; superoxide dismutase (SOD), catalase (CAT), ascorbic peroxidase (APX) enzymes in the antioxidant defense systems are analyzed on 12 lines and two cultivars of pea seedlings which are specified as tolerant against cold. Besides the relationship between the protection mechanisms from abiotic stress conditions (change on the antioxidant enzymes) on the pea plant is tried to be revealed. In terms of macro and microelement accumulation, considering control plants, the effect of the low-temperature application is not observed in Mn, Mg, Cu microelements. However, approximately ten times decrease is observed on Fe and Zn accumulations. It is also observed that there is not a change in K and Ca accumulation of the plants considering control

plants. It is seen that with the low-temperature application, there are significant increases in CAT, APX and SOD enzyme activities which are among the antioxidant enzymes of the plants (Togay et.al., 2016). In the cold stress-resistant pea genotypes, biochemical, physical properties or physical defense mechanisms created by plants against cold stress have been tried to be revealed. If cold harm was examined, Melrose, Sahin, Granger, 4053 x Melrose, 4053 x Hadim, Sahin x Hadim and 3057 x Melrose; if peroxidase content was examined 3057 x Melrose, 4053 x Melrose, 3029 x Melrose, 3029 x Granger and 4053 x Hadim, when superoxidase content was examined, 3053 x Melrose, Sahin x Hadim, 4053 x Melrose, Sahin x Melrose and 3029 x Melrose and when proline content examined 3031 x Granger, 3055 x Melrose, Ultrillo, 3057 x Hadim and Sahin x Hadim the genotypes were first. As a result, when the effects of cold stress on the enzyme activities in the leaves of pea genotypes are considered, the most durable genotypes are 3031 x Granger and 3055 x Melrose. These come forward as genotypes that can be used in subsequent studies to breed for cold resistance (Tekin and Ceyhan,2020). Similarly, the 127 genotypes were treated by single cold stress (2/2 °C day/night temperature (DT/NT)) and 42 genotypes were treated by either a single episode of cold or heat (38/30 °C DT/NT) stress or a combination of both at photosynthetic photon flux density of 250 mol m⁻² s⁻¹. Chlorophyll fluorescence was used to detect the tolerance of faba beans to low and high temperatures. The maximum quantum efficiency of photosystem II (PSII), Fv/Fm, revealed pronounced differences in cold tolerance among the faba bean genotypes. The 42 genotypes were clustered into four groups according

to cold and heat stresses, respectively and the susceptibilities of faba beans under temperature stress could be distinguished. The combination of cold and heat stresses could aggravate the damage on reproductive organs, but not on the leaves, as indicated by the Fv/Fm. These results confirm that the use of Fv/Fm is a useful approach for detecting low and high-temperature damage to photosystem II and to identify tolerant faba bean genotypes, however, the results also indicate that the geographical origin of the genotypes could not directly be used to predict climate resilience. These sources of cold- and heat-tolerance could improve the temperature tolerance of faba bean in breeding programs (Zhou et. al., 2018). Yaşar et al. (2017) determined the developmental performances of 18 bean genotypes collected from various regions of Anatolia and belonging to the species *Phaseolus vulgaris* L., according to their organs under chilling stress. It was found that bean plants had differences in their root, stem, leaf weights and leaf counts between genotypes compared to controls. On the other hand, low temperature showed high amylase as well as catalase activity in dry bean. Room temperature showed a good response against few parameters but not in enzyme activity (Srivastava et.al., 2015).

THE OTHER STRESS FACTORS

One of the methods of struggle against adverse environmental conditions is to provide tolerance against stress by using molecules that have the characteristics of plant growth regulators (Ashraf and Foolad, 2007). It has been found that plants stimulate MEL production to cope with toxic environmental stressors in adverse environmental conditions

such as extreme cold, sunlight, soil pollution caused by heavy metals and chemicals (Arnao and Hernandez-Ruiz, 2009,2013; Byeon and Back 2014; Tal et.al., 2011). MEL, which has recently been accepted as a plant growth regulator, acts as an antioxidant in increasing tolerance against many stress factors (high and low temperature, drought, salt, alkalinity, chemical pollutants, etc.). It has been reported that the external application of MEL to bean seeds in moderately alkaline soils can be used to increase tolerance to stress (Yakupoğlu, 2020). It is known that heavy metals have toxic effects on various mechanisms in plants. In response to these toxic effects, it becomes a necessity to talk about transcription factors in the formation of reactions to stress conditions and in the adaptation process to these conditions in order to know the heavy metal toxicity tolerance limits of the plants, it is necessary to know the data on the metal type and amount, the degree of damage and also the damage formation process. Knowing and measuring these scientific data is very important for the development and vitality of plants. CAMTA and YABBY genes (transcription factors) were selected and the gene expression profiles of two different bean cultivars (Akman-98- Önceler-98) exposed to lead (Pb), copper (Cu) and cadmium (Cd) stress were compared. Significant differences occurred in the expression of target genes after application of different concentrations of copper (Cu), cadmium (Cd), lead (Pb) stress in leaf tissues of bean varieties compared to control. It was determined that the increase in lead (Pb) concentration was not very effective on CAMTA gene expression in Akman-98 cultivar, while copper (Cu) concentration caused an increase in gene expression level in Akman-98 cultivar and a

decrease in Önceler-98 bean cultivar. It has been reported that in the samples subjected to cadmium (Cd) stress, an increase in expression of both genes was observed in Akman-98 bean cultivar, while a decrease in the expression level of the YABBY-7 gene and an increase in the expression level of the CAMTA-4 gene occurred in the cultivar Önceler-98 (Kocaman, 2019).

TCP proteins are a family of genes that contain important transcription factors that have roles in plant development and growth, such as leaf growth, organogenesis, and lateral branching. Chromium (VI) in high concentrations prevents seed germination, changes the structure of the chloroplast and cell membrane, causes the emergence of reactive oxygen species, and has harmful effects on animals and humans. The gene expressions of Pvul-TCP-2, Pvul-TCP-3, Pvul-TCP-13, Pvul-TCP-20, Pvul-TCP-27 in bean plant under chromium stress were analyzed at the mRNA level. It has been argued that the TCP gene family plays a role in the defense mechanism against chromium (VI) heavy metal stress in the bean plant. (Keskin, 2019). Expression levels of stress-related genes were investigated in chickpea (*Cicer arietinum* L.) leaves exposed to copper heavy metal at different times and concentrations. The amounts of hydrogen peroxide (H₂O₂), which is one of the reactive oxygen species (ROS), and malondialdehyde (MDA), which is an indicator of oxidative stress in the cell, were determined by taking samples from the leaf tissues of the plant. In addition, the changes in the expression levels of cellular antioxidant enzymes superoxide dismutase (Cu-Zn/SOD) and catalase (CAT) and

metallothionein (MT2) protein, which has antioxidant properties, were determined according to the expression level of the actin (ACT) gene selected as the house-keeping gene. It was determined that there was a very stable increase in MDA and cellular H₂O₂ concentrations depending on the increasing time and concentration, while the expression levels of Cu-Zn/SOD, CAT and MT2 were higher than the control at all times and concentrations (Öztürk, 2019).

CONCLUSION

Like all living things, legumes are affected by abiotic stress factors in different ways. In the face of these factors, they develop responses at the morphological, physiological and genetic levels. In this study, some of the studies conducted in the last 20 years on stress factors such as drought, salt, cold, alkalinity and heavy metals in food legumes are discussed. It is obvious that abiotic stress conditions will increase with global climate change. Studies that have been done or will be done especially at the physiological and genetic level will bring new approaches to the cultivation of grain legumes, as well as other plants. While suggesting some measures to be taken against stress factors, it will lead to the determination of priority issues in the breeding studies to be carried out.

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CHAPTER 3

ABIOTIC STRESSES EFFECTS ON GRAIN COMPOSITION AND QUALITY IN EDIBLE LEGUMES

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1. INTRODUCTION

President of the International Pulses Confederation Cindy Brown (2016) describes legumes as a superfood in her speech emphasizing that the role of legumes in the sustainable food systems of the future has increased in our age marked by climate change and population growth. They are the plants of the future, and he says that legumes are vital to the sustainable food systems needed to feed the world's population, which is expected to reach 10 billion by 2050. She also reports that environmentally sensitive farmers prefer legumes to reduce their carbon footprint. While researchers working on legumes have been trying to express the richness and environmental friendliness of legumes, especially in terms of nutrition, they are very happy that the public has now realized this. As human knowledge increases, their demands also increase and they expect the food they will consume to be healthier. For this reason, the food sector carries out intensive studies and studies that reveal the importance of food in terms of health are increasing day by day. In recent years, studies have intensified not only productivity and cultivation techniques but also their quality and beneficial properties for legumes.

In addition to carbohydrates, the main energy source, proteins that work in all vital functions are obtained from animal and vegetable sources. While there is around 25% protein in vegetable protein sources, this ratio is 18-20% in meat, which is an animal protein source (Savur and Ceyhan, 2011). The amount of protein consumed daily in the world is 70.9 g/person on average, 85.0 g/person in Turkey, 104 g/person in

developed countries and 61 g/person in developing countries (Ceyhan and Şimşek, 2021). Although protein is present in all plants, the plant groups containing proportionally more protein are legumes.

Agriculture is the most important sector in the future of humanity as it was in the past. Unfortunately, while human beings developed and modernized, they misused natural resources, and other plants, animals, soil, air, etc. put it in the background. This situation has created and continues to create problems that are difficult to solve. Polluted soil, water and air, that is, changing environmental conditions cause many changes in the cultivation of agricultural products. When the environmental conditions change to such an extent that they affect the normal growth and development of a plant negatively, the situation that occurs in the plant is called stress. In other words, it is defined as external factors that have negative effects on the plant.

In addition to the importance of legumes in terms of improvement of agricultural areas, their quality features in terms of health have made them remarkable products all over the world. In this chapter, the quality characteristics of legumes and their changes under abiotic stress conditions will be examined.

2. QUALITY COMPONENTS OF GRAIN LEGUMES

Legumes are very nutritious and to play a very important role in a healthy life. The high protein and fiber ratio helps to fight obesity while providing a feeling of satiety. Carbohydrates that include in legume, they provide a longer-lasting energy supplement instead of the short-

term and immediate energy provided by simple sugars. In terms of intestinal health, oligosaccharides and resistant starch reduce the risk of diabetes, heart disease and even some types of cancer, thanks to their newly discovered antioxidant content, vitamins, high fiber content and low-fat rates (Cummings et al., 2004). Epidemiological studies reported an inverse association between the consumption of legumes and the incidence of age-related diseases (Costantini et al., 2021). This trend could be attributed to the presence of antioxidant compounds, especially phenolic and flavonoid compounds.

Table 1. Major components of edible legume crops

	Bean	Chickpea	Lentil (green)	Lentil (red)	Pea	Faba bean	Cowpea
Energy (kcal)	281	334	299	322	309	323	313
Protein (g)	21.75	18.56	23	25.81	19.82	23.07	22.88
Carbohydrate (g)	29.42	41.35	36.62	41.94	42.98	46.66	43.74
Oil (g)	1.35	5.33	0.92	1.57	1.15	1.6	1.01
Starch (g)	21.64	30.98	32.95	40.95	42.98	23.07	
Total fiber (g)	32.17	23.03	25.99	18.67	23.65	14.71	18.89
Ca (mg)	141	99	64	26	125	54	87
Fe (mg)	4.71	5.92	7.77	5.87	6.79	6.1	7.43
P (mg)	367	397	415	416	295	488	386
B1 vit (mg)	0.796	0.572	0.159	0.809	0.709		
B2 vit (mg)	0.181	0.164	0.148	0.177	0.186		
Niasin (mg)	4.141	3.146	4.613	2.68	3.813	2.83	3.001

(TURKOMP, 2021)

The quality of edible legumes primarily bases on grain composition including protein, carbohydrate, dietary fibers, oil, sugars, fatty acids, minerals and vitamins. Grain legumes may include the fifteen essential nutrients required by the human body in different concentrations (Wang et al., 2003). Phosphorus, potassium, calcium, zinc, iron, boron and manganese are the major minerals in grain legumes and deficiency of

them may lead to human malnutrition and/or health. In terms of nutritional value in edible legumes, quality components can be examined in two groups as components that positively affect nutrition (high protein and low-fat content, vitamins, mineral substances, dietary fibers) and components that negatively affect nutrition (antinutritional factors).

2.1. Proteins and Amino Acids

Proteins have a wide range of functions in all vital activities in living cells. Proteins perform and control many physiological events in all living things such as control of growth and differentiation, coordination of activities, enzyme activity, collagen tissue formation and support and protection, transport and storage, perception and movement (Hao et al., 2011). Sources of protein are animal, plant foods and fish. For his feature legumes are the most important group in the herbal foods. These plants are also called protein plants. Edible legumes are inexpensive and high-quality herbal protein sources. They contain an average of 20-25% protein in their seeds that this rate is about twice higher than cereal grains (Table 2). This rate varies depending on the type, variety and growing conditions of the legume.

Proteins are polypeptides formed by the covalent bonding of amino acids to each other in a characteristic straight chain in a certain type, in a certain number and a certain sequence. While plants can use inorganic nitrogen sources such as ammonia, nitrate, and nitrite, humans and some animals require most of the amino acids to make their body proteins. All living cells contain proteins. In humans, amino acids are

required for protein synthesis (Hao et al., 2011). The main component of proteins is amino acids that are divided into three groups as essential, semi-essential and non-essential. An essential amino acid, or indispensable amino acid, that cannot be synthesized from scratch by the organism fast enough to supply its demand, and must therefore come from the diet. They are valin, leusin, izoleusin, fenil alanin, triptophan, treonin, methionine, lizin, histidin (in child) (Murray et al., 2014). Sulfur-containing amino acids that are low in the proteins of legume seeds are cysteine, methionine (< 2%) and tryptophan (< 1%) (Sarioğlu and Velioglu, 2018). Methionine is a supplier of sulfur. Without enough sulfur, the body cannot produce and use some antioxidant nutrients. Therefore, Methionine is a very valuable nutritional compound that brings numerous benefits to your body. Tryptophan is considered the cornerstone of numerous life-giving biomolecules such as enzymes, structural proteins, serotonin, melatonin, and neurotransmitters.

The useful protein contents in cooked beans vary according to preparation methods such as soaking and temperature treatments. These methods cause a decrease in protein content and increase protein digestibility (Sarioğlu and Velioglu, 2018). Sarioğlu and Velioglu also reported that methionine was 0.23% in raw beans, 0.08% in cooked beans, and 0.17% in 3 hours at 65°C.

Khattab et al. (2009), investigated the effects of some physical applications on the nutritional quality of legume seeds. In the study, cowpea, beans and peas grown in Canada and Egypt were used. They

reported that tryptophan, cysteine and methionine amino acids were the lowest in all legumes

The protein should not be high but should be highly digestible. The digestibility rates of the proteins of legumes vary between 72-97% according to the species (Table 2). The amount of protein taken into the body that can be converted into body proteins is called the actual protein value.

Karayel and Bozoğlu (2015) investigated the differences among local peas lines and four varieties that sowed at different sowing dates under Samsun conditions. According to results, raw protein ratio and tryptophane quantity exhibits significant differences depending on sowing times genotypes. The raw protein ratio and tryptophane quantities changed between 17.73-28.36% and 1936.42-3119.74 ppm, respectively in winter sowing. In spring sowing, raw protein ratio ranged from 19.29 to 27.17%, tryptophane quantities from 2231.05 to 2746.44 ppm. Light green seed colored, wrinkle seed line gave the highest value.

Table 2. Crude protein, its digestibility, methionine and triptophane content in the same food

	Protein %	Digestibility %	Methionine mg/g*	Tryptophane mg/g*
Bean	23	74.6*	12-15	11.9-43.1
Faba bean	26	72.1*	2-8.2	9.5-10.6
Chickpea	19	66.4*	14-18	10-15.2
Cowpea	22	75.5*	10.9-14.2	1.3-12
Lentil	26	73.1*	8.5	9-14.6
Pea	25	82.4*	2.8-11.4	2.7-14.4

Meat	19.5	94±3		
Chicken	23.4			
Egg	12-16	97±3		
Fish	18-24	94±		
Milk and its products		95±3		
Wheat	11	86.8*	15.4-16.1	10.2-12

2.2. Carbohydrate and Starch

Total carbohydrates in legumes can range from 24 to 68%. There is an expression in Turkey: Beans are both meat and bread for poor people. Because beans are rich in both protein and carbohydrates. In other words, the person who eats legumes both gets her stomach full and her body. Carbohydrates are made up of sugar, starch, and other polysaccharides. Starch is the most important part of legumes. Legume starch is more digestible than the starch of potatoes and corn. But less digestible than cereal starch. Starch is divided into 3 groups as rapid and slow digestible, resistant starch. There is increasing interest in the health benefits of rapidly digestible starch content of legumes (Sarioğlu and Velioglu, 2018). Protein and oils cause slower digestion of carbohydrates and increase insulin secretion. While starchy foods are digested faster on their own, it is digested more slowly when consumed with protein (Yılmaz, 2019).

Karayel (2012) studied with 44 lines and 4 varieties of peas. The starch ratio was found to be 33.88% in winter sowing and 32.41% in early spring sowing date, and this difference was found to be statistically significant. In the winter sowing, starch ratio of genotypes was found

between 24.52-38.78 %. Bezmen (2019) studied 15 genotypes of faba bean sown in winter on the Samsun conditions and reported starch ratio changed between 31.12-38.98 %.

2.3. Dietary Fibers

Dietary fibers refer to indigestible components in the small intestine. It contains polysaccharide components such as cellulose, hemicellulose, pectin, and non-carbohydrate components such as gum, resistant starches and lignin. Total dietary fiber (TDF) consists of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) which have an important place in nutrition. Soluble fiber is generally associated with a reduction in cardiovascular diseases, while insoluble fiber has a protective effect against bowel cancer. Studies show adequate fiber intake as an essential factor in the prevention and control of chronic diseases. A daily intake of 10-15 g of soluble fiber has been shown to reduce total cholesterol and LDL-cholesterol in the blood. Soluble fiber, which is thought to have a cholesterol-lowering effect, has also been found in foods containing beans. It has also been proven that a higher intake of dietary fiber has a blood pressure lowering effect. In some studies, researchers agree that increasing the intake of dietary fiber increases the feeling of satiety after a meal and reduces the subsequent feeling of hunger. This feature of dietary fiber can contribute to reducing body weight (Sarioğlu and Velioglu, 2018).

Grain legumes are an excellent source of soluble dietary fiber. They contain approximately 3-7% soluble fiber. Numerous studies have shown that soluble dietary fibers have beneficial effects on

cardiovascular disease in humans, particularly by lowering both total serum and low-density lipoprotein (LDL) cholesterol levels (Glore et al., 1994). In addition, clinical studies have shown that it is beneficial for the second type of diabetes because it reduces postprandial blood sugar, insulin amount and lipid level in blood serum (Tabatabai and Li, 2000). Grain legumes also contain insoluble dietary fiber, which is beneficial for intestinal health due to its laxative (laxative) effect and has an average of 11%. Consumption of insoluble dietary fiber has a reducing effect on the risk of bowel cancer (Herbert et al., 1995). Nutrition with dietary soluble and insoluble fibers has shown positive effects on weight loss (Anderson and Bryant, 1986).

2.4. Antioxidants

Humans obtain their energy needs as a result of reactions controlled by oxidative metabolism, that is, aerobic metabolism, which needs oxygen. While oxygen is reduced to water for energy during oxidative metabolism, a very small amount of it turns into harmful substances that have lost their electrons, called "free radicals". In the meantime, the structure and functions of molecules may change, cell damage may occur. The resulting damage is called "oxidative damage". Free radicals have direct effects on cell growth and development. Compounds or systems that prevent damage caused by oxidation by preventing free radical formation, stopping or reducing the activity of free radicals are called antioxidants (Singh and Singh, 2008). Antioxidants function by scavenging free radicals or converting them to a weaker new molecule, interacting with free radicals and reducing their activity, breaking or

repairing the reaction chain by binding free radicals to themselves (Velioglu et al., 1998).

Antioxidants are divided into two large groups: enzymatic and non-enzymatic (endogenous or exogenous). Among the endogenous antioxidants, it is possible to count enzymes, hormones and various acids. In exogenous antioxidants, vitamins, minerals, polyphenols, carotenoids occupy a significant place.

Flavonoids are polyphenolic compounds found in foods of plant origin. According to studies, more than 4000 flavonoids have been identified (Hournan et al., 1996). Phenolic compounds only effectively prevent oxidation in food systems and also act as protective factors against oxidative damage in the human body (Bartolome et al., 1997). The synthesis, oxidation and amount of certain phenolic compounds vary depending on the stress type. Phenolic content changes with light and light intensity.

Antioxidants, which are secondary metabolites, are in herbs, flavonols; flavonones in vegetables, fruits and beverages; in fruits, catechins; in fruit and beverages, anthocyanidins; fruits, isoflavones are found in vegetables and legumes. Hournan et al. (1996), flavonols and flavones at low (<10mg/kg or <10mg/lt) in legumes, medium (<50mg/kg or <50mg/lt) in beans and high (>50mg/kg>50mg/lt) in beans have reported that. It has been reported that the antioxidant effect is high in herbal products such as spices, herbs, cocoa shells, coffee beans, oats, tea, beans, broad beans, peas, tomatoes, cranberries, especially onions and peppers, olive leaves and soybeans (Sherwin, 1990).

3. EFFECTS OF ABIOTIC STRESS ON QUALITY COMPONENTS IN GRAIN LEGUMES

Environmental stress factors induced drought, salinity, temperature extremes and heavy metals adversely affect grain protein, starch, amino acids, fats and vitamin contents in grain legumes, therefore, they cause decrease crop quality. Stress in plants disturbs physiological and biochemical processes such as photosynthesis, nutrient uptake, ion and hormonal balance, thereby inhibits morphological growth, grain yield and quality (Araujo et al., 2015; Farooq et al., 2017; Ceritoglu et al., 2020; Ullah and Farooq, 2021).

Yazdi-Samadi et al. (1977) stated that salt stress adversely influences protein and oil contents of soybean grain. Ghassemi-Golezani (2010) reported that protein content in chickpea grain decreased under salinity stress due to disturbing of N metabolism and NO_3^- uptake from the soil. Similarly, reduction of protein content in grain was observed in faba bean, cowpea, bean, black gram and lentil depending on salinity stress (Ashraf, 1989; Silveira et al., 2001; Dantas et al., 2007; Misra and Saxena, 2009; Ali et al., 2013). Amira and Qados (2010) determined that carbohydrates, polysaccharides, amino acid and protein content decreased in mung bean grain with increasing salinity level due to osmotic stress, low N uptake, nutritional imbalance, reduced photosynthesis and specific ion toxicity. Moreover, increasing salt stress led to reducing nitrogen, phosphorus and potassium concentrations in grain while it provided to rise magnesium, calcium, sodium and chloride content. As a result, salinity stress has a pivotal

role in quality reduction in legume grain. Bandoğlu (2001) stated that salinity stress changes antioxidant enzyme activities such as CAT, SOD, APX and glutathione reductase, cell membrane stability, lipid peroxidation, malondialdehyde, proline and H₂O₂ content, and physiological changes of lentil seedlings. Lengths and weights of shoot and roots decreased with increasing salinity stress. Lipid peroxidation rised in leaves under NaCl stress. An increase was observed in proline content in plants that grown under salt stress. Ercan (2008) investigated the impacts of salinity stress during early-seedling stage of lentil and observed the effects of stress on physiological, biochemical and morphological growth. Salinity stress caused a major decrease in water content, seedling weight and length, chlorophyll fluorescence and antioxidant enzymes activities. Ercan pointed out that Glutathione reductase, Ascorbate peroxidase and proline have a pivotal role in antioxidant defense systems against salt stress. Hassanein et al. (2012) investigated the effect of different concentrations of NaCl (100, 150, and 200 mM) on cell membrane stability, photosynthetic pigment and carbohydrate contents, antioxidant enzymes activities, glutathione contents, and lipid peroxidation (malondialdehyde content) in faba bean (*Vicia faba*) leaves. The results revealed that salt-stressed bean plants treated with stigmasterol had an increased membrane stability index, and photosynthetic pigment and carbohydrate contents compared with salt-stressed plants untreated with stigmasterol. The soluble and insoluble sugars, and total carbohydrates reduced with increasing salinity stress compared with optimum conditions. Seed priming with stigmasterol led to significant increases in soluble and insoluble sugars,

and total carbohydrates in broad bean. The maximum total carbohydrate content was estimated to be increased by 150.6% in plants treated with 150 mM NaCl + stigmasterol compared with that of the reference control (Hassanein et al., 2012).

Drought stress is another major restricting factor on grain quality. Triboi et al. (2003) indicated that although protein quality of seed highly depends on genotypic characters, environmental factors may also affect N accumulation. Drought during grain filling time delays the seed-filling rate and decreases the filling duration to limit seed size (Sehgal et al., 2017). Drought stress may lead to membrane damage (Awasthi et al., 2014) and the photosynthesis process (Anjum et al., 2011) due to stomatal conductance or some other associated mechanisms. Drought stress distracts mineral uptake (Gunes et al., 2006) and sharply reduces nitrogen fixation in legume plants (Furlan et al., 2017; Marinkovic et al., 2019; Nadeem et al., 2019). Thus, it causes a decrease in assimilate production and mobilization to developing seeds in various crops (Mafakheri et al., 2010; Zare et al., 2012). It particularly causes inhibits the accumulation of protein, oil and carbohydrates (Mansourifar et al., 2011). However, the response of different legume species to drought stress is changeable depending on drought level and growth period. Moreover, whereas the protein content of legume grain may increase, it declines in some cases. Behboudian et al. (2001) denoted that a mild water deficiency may promote protein assimilation during the flowering stage in chickpea. According to the study results, mild drought promoted the protein content of grain in

mung bean and chickpea up to 6-21%. However, severe drought inhibited protein content by 19-35% in lupins (Khalil and Ismael, 2010). These differences are thought to be caused intensity and duration of stress exposure on plants, and relative to seed dry weight. The increased protein content under mild drought is linked to altered C-partitioning which changes the C/N ratio, to favor more N-assimilation (Sehgal et al., 2018). Thus, these studies indicate crop-specific effects of drought stress on proteins and minerals. On the other hand, Ghanbari et al. (2013a) claimed that drought stress reduced phosphorus, nitrogen, iron and zinc contents, thereby, leads to a decline in the total proteins in the common bean grain. In a general perspective, severe drought inhibits cell division and starch granules, reduces carbohydrates, oil, sugar contents and total nitrogen assimilation (Sehgal et al., 2018). The various researcher reported that drought stress during the different development processes, particularly at the seed filling stage, causes corruption of grain quality through reducing protein content in bean (Ghanbari et al., 2013b), chickpea (Nayyar et al., 2006), cowpea (Carvalho et al., 2019), lentil (Choukri et al., 2020), faba bean (Ouzounidou et al., 2014), pea (Karatas et al., 2012), soybean (Maleki et al., 2013), black and green gram (Baroowa, and Gogoi, 2015). Çevik (2009) investigated the effects of ascorbate and glutathione on antioxidant system of chickpea cultivars which have different tolerance to drought conditions *Cicer reticulatum* AWC611 (drought tolerant) and *Cicer arietinum* ILC8617 (drought sensitive) under drought stress conditions. Chickpea plants were grown for 35 days, after that plants were exposed to drought stress for 10 days. For first three days of the

drought stress, ascorbate (12,5 mM and 25 mM) and glutathione (10 mM and 100 mM) were applied to both groups which drought exposed and not exposed. Drought stress enhanced antioxidant enzymes activities and antioxidant concentrations in all groups (except catalase). Applications of ascorbate and glutathione (GSH) increased ascorbate peroxidase activity, ascorbate and GSH concentration. Superoxide dismutase and glutathione reductase activities decreased on the leaves of both genotypes. Enzymatic and non enzymatic antioxidant capacity of drought-tolerant AWC611 was determined higher than drought-sensitive ILC8617. It is determined that under drought stress the response of AWC611 to exogenous antioxidant applications was better than ILC8617. Abid et al. (2017) stated that water deficiency adversely affects chlorophyll fluorescence. The SOD, CAT and GPX activities increased under drought stress. Dadasoglu et al. (2021) determined that Nitric oxide (NO) may protect the plants which are exposed oxidative stress with various biological ways. According to results, exogenous NO enhanced electrical conductance, reduced H₂O₂ and MDA content, increased chlorophyll content and nutrient uptake.

Heat stress leads to corruption in grain quality via reducing total sugar, protein, various amino acids, soluble protein, carbohydrates and oil content. The primary damage of high temperature is based on denaturation of protein structure; therefore, disturbed proteins cannot properly work and this situation causes decrease grain quality (Lipiec et al., 2013; Liu et al., 2019). Gaur et al. (2015) determined that heat stress restricts electron flow and photosynthesis, interrupted metabolic

pathways, damages seed formation and growth, thereby, it decreased grain yield in chickpea. Heat stress disrupts sucrose metabolism in leaves and breaks sucrose supply for seed development in chickpea (Kaushal et al., 2013), mung bean (Kaur et al., 2015). In addition, declining chlorophyll in the leaf strongly coordinates with contents of non-structural carbohydrates and nitrogen as well as their remobilization efficiencies (Tahir and Nakata, 2005). Golombek et al. (1995) indicated that heat stress caused decreases in total sugar, starch and protein by 24.5%, 53% and 19.6%, respectively, compared with optimum growth conditions. Wolf et al. (1982) stated that increasing temperatures at the growing medium led to a decrease in sucrose content of soybean seed by 56%, while it caused an increase in oleic acid by 256%. Kaushal et al. (2013) observed that heat stress caused a decrease sucrose content up to 9% in chickpea grain. In conclusion, heat stress lead to senescence of leaves, reducing net photosynthesis, chlorophylls, hastens seed filling, corrupts sucrose-starch conversion and leads to loss of sink activity to decrease the seed weight and quality (Sehgal et al., 2018).

Higher concentrations than optimum levels of heavy metals such as Pb, Cd, Cr, Hg, Zn, Cu, As and Ni, in the rhizosphere both reduces grain yield and quality, also they may accumulate in grains, thereby, leads to health concerns (Lebrazi and Fikri-Benbrahim, 2018; Karimi et al., 2021). Many different researchers reported that heavy metals, particularly Cd and Pb, accumulated legume grains at a higher concentration that causes health problems (Athar and Ahmad, 2006;

Wang et al., 2006; Alyemeni and Almohisen, 2014a, 2014b). Accumulating heavy metals exhibits differences depending on heavy metal species, host plants and parts of the host plants. According to the examinations, bean accumulates heavy metals, particularly in grains. Whereas pea seed accumulates iron and zinc, lentil seed involves low concentrations of lead. Soybean leans accumulation of toxic heavy metals than other legumes or cereals (Lavado et al., 1995; Sajwani et al., 1996; Angelova et al., 2003; Shute et al., 2006). Alyemeni and Almohisen (2014a) were examined by quantifying the trace element Cu, Mn, Pb and Zn protein concentrations in leaves, pods and grains pea, faba bean, soybean and cowpea species. They indicated that heavy metal stress based on the traffic, industrial activities and population density leads to heavy metal accumulation in different parts of grain legumes, thereby, causes reduce grain quality and increase health problems. Athar and Ahmad (2006) metal uptake by legume grains seemed to be directly regarded to the concentration of heavy metals and is effective on grain quality. Besides, different heavy metal stresses cause a decrease in protein, oil and fatty acid concentrations in different legume plants (Farooq et al., 2018). Arsenic (As) being a toxic metalloid adversely affects plant growth and yield, as well as poses severe risks to human health. Hydrogen sulfide (H₂S) has emerged as a vital signaling molecule regulating key plant growth processes under stress conditions. However, to date, little information is available regarding the role of H₂S in mitigating As toxicity in pea plants. Hence, H₂S reduced oxidative damage and promoted the growth of pea plants under As stress, suggesting an important role of H₂S in plant priming.

The dark period is another major stress for the plants and causes significant changes in plant metabolism. In the study of Akgül (2010), the antioxidant enzyme activities and the amounts of hydrogen peroxide, malondialdehyde and total phenolic compounds in seedlings of bean were investigated. It was seen that the antioxidant enzyme activities, hydrogen peroxide, malondialdehyde, and proline contents in etiolated seedlings were increased more than both the control and de-etiolated seedlings. Total phenolic compounds were not determined in etiolated seedlings although they were found in both control and de-etiolated seedlings. It was observed that the light increased the synthesis of phenolic compounds in bean seedlings. Humaira et al. (2020) indicated that light-dark cycle has a pivotal role in lifespan of plants. The supplementation of Cu-QDs for 72 h under dark conditions promoted the germination of chickpea seeds. The photosynthetic pigments were also enhanced depending on Cu-QDs treatments under optimum and dark conditions.

Arya et al. (2011) denoted that manganese is a major element for broad bean and they studied on Mn accumulation in different organs, chlorophyll and proline content, and PX activity. Manganese level in root and shoot tissues rised with increasing Mg doses and it was higher in roots compared with shoots. However, there was about 2 fold increase in total glutathione content at 40 microM than the basal level and further declined to 21.65 micrograms (-1) fresh wt. at 160 microM Mn. The results shows that broad bean can cope with manganese

exposure throughout enhanced production of antioxidant enzymes activities.

5. CONCLUSION

Legumes are preferred by consumers due to their valuable nutritional characteristics (high protein, digestible/indigestible carbohydrates, polyphenols, antioxidants, starch, etc.) and low cost. Also, they have a preventive effect on coronary and cardiovascular diseases due to the high amount of antioxidants. Thus, legumes have a pivotal role in diets and human nutrition. However, various environmental stresses cause decrease grain quality and threats animal and human health by the accumulation of heavy metals in different parts of legume plants.

Unfortunately, breeding studies for yield improvement and especially quality characteristics in legumes have not been as diverse and numerous as in cereals. Although this situation is a little bit more for culture under biotic stress conditions, it also applies to the effect of abiotic stress conditions, especially on seed quality. This review concluded that this area is as lacking as possible and that legume researchers should focus their attention in this direction. Therefore, practices on mitigation of environmental stresses on grain legumes are required to study with new and original approaches to protect grain quality and food security.

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CHAPTER 4

DROUGHT STRESS: IMPACTS ON GRAIN LEGUMES, WAYS TO MITIGATE NEGATIVE EFFECTS AND ITS MANAGEMENT

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INTRODUCTION

Food grain legumes are the best source of protein both for human as well as for livestock. Pulses are considered together with cereals for providing food security (Akibode and Maredia, 2012). Crop rotation of non-leguminous crops with leguminous crops improves soil fertility, reduces soil erosion, increases soil carbon nitrogen contents and had a very positive impact on subsequent crop (Bagayoko et al., 2000). Encouraging legume cultivation in less developed countries could help to achieve one of the sustainable goals that is 'eliminate hunger' set by Food and Agriculture Organization, in combination with improve poor people income, provide healthy food with clean and safe environment (Abate et al., 2012). Crop diversification of leguminous crop with non-leguminous crops is a way to increase the profitability and sustainability of agricultural production systems (Hatfield and Karlen, 1994). World demand for pulses has increasing day by day not only in poor countries, but also in the developed countries due to health risks sourced from animal protein consumption is more widely recognized given the trend towards healthy dieting by consuming pulses.

According to FAO report published in 2018, the world pulses production was about 92.4 million metric tons that is 63% higher than two decades back production. That is very positive trend in term of food security but at the same time world population is increasing day by day. If world population continues to rise with current pace, it may add forty percent more people in this world by 2050. This world

already having limited resources and will face big challenge to produce more food almost seventy percent or even higher to hundred percent by 2050 (Bruinsma, 2009). Producing high quantity of food is a big challenge and it becomes further complicated by the hard competition for land resources, water availability for agriculture, fisheries, livestock, industry and urban development (Postel, 2000). Such factors force agricultural crops to the marginal lands, where already water shortage is one of big challenges to grow crop. Uneven or untimely rainfall further worsens the position of agriculture in these marginal areas. These factors significantly negatively influence food security in such natural rainfall dependent areas. There are some natural variations exist in plants for drought stress (DS) tolerance depending upon extent and severity of water sourced stress, plant species, and also plant growth stages (Singh et al., 2012).

Legumes are mostly grown in areas where no any established irrigation system is available to irrigate the crops so untimely or limited rainfall may cause DS at various growth stages. The extent of crop performance loss depends upon severity of moisture scarcity and the crop species grown in particular area. Chickpea, broad bean pigeon pea, groundnut, soybean and lentil are generally exposed to terminal DS while pigeon pea and groundnut are also encountered intermittent DS resulting in high yield losses (Subbarao et al., 1995). Cowpea (*Vigna unguiculata* L.) is a more preferable pulse generally cultivated in semi-arid agricultural regions where terminal DS events frequently causing yield loss. Soybean (*Glycine max* L.) is sown at the

beginning of spring season or later in United States, South part of US and China. In those area soybean crop experience relatively, less DS compared with other legumes demand higher water up to 700 mm rainfall (Dogan et al., 2007). Common bean modifies its growth period according to the availability of water ranges from 60-120 day (FAO, 2012). Common bean is highly responsive to irrigation and DS spells. DS at reproductive growth stage adversely affects seed yield and quality (Upreti et al., 1997). DS not only disturb plant performance but also cause huge economic losses due to reduction in yield per unit area. The aim of present book chapter is to cover the effect of DS, how much losses it causes to legume crops and also briefly describes the different management strategies to overcome DS in different legume crops.

Grain legumes thought to be drought tolerant but what the reality is?

DS may be described as the condition when the plant cannot manage water requirement after that via present water in the soil, leading to decreased tissue water content in plants, and thus largely modifying plant metabolism according to soil moisture availability. Moisture stress is one of the most essential challenges encountered by legume growers throughout the world (Nadeem et al., 2019). Water is basically needed to grow any of crops to secure economic profit. Grain legumes have great potential to withstand under limited water availability (Savita and Singh, 2020). Generally, legumes especially chickpea is grown in areas with less availability of resources such as

water, nutrients than other crops. Although legumes require less water as compare to other field crops but DS during early growth and/or terminal growth stages resulted significant yield loss. Legume crops especially chickpea is one of best option for the areas having uneven sandy soil and if crop receive two effective rains during its life cycle are enough to get high yield in rainfed areas. Legumes are promising in their ability to withstand under DS because of nitrogen fixation by the interaction with soil microorganism's and arbuscular mycorrhiza (Lodeiro et al., 2000). Although some research finding represent that nitrogen fixation might be reduced due to the low water availability (Serraj et al., 1999). Many research investigations represent that genotype respond differently under the prevailing condition of DS (Smith et al., 1988). Plant water status can be represented by using different variable in term of leaf water potential (Ψ_w), relative water content (RWC) (Hsiao, 1973) or other parameter showing water fluxes within the plant fraction of transpirable soil water (Christophe et al., 2011). In field pea, the water potential has been observed around $\Psi \sim -1.2$ MPa, and fraction of transpirable soil water near $\sim 0.1-0.2$ MPa under DS condition (Lecoeur and Guillioni, 1998), although this pressure may differ among plant species and genotypes (Ladrera et al., 2007). Daryanto et al. (2015) informed that legume crops having high osmotic adjustment under DS has ability to maintain high yield under low water availability. The ranges of yield reductions in legumes are extremely unstable because it relies on severity and timing of DS. (Emam et al., 2010). Green gram and groundnut exhibited the lowest

yield reduction as shown in table 1. The detail of yield reduction in different legume crops is given table 1.

Table 1: Yield Losses Due to Drought in Various Legumes at Different Growth Stages

Crop name	DS stages	(%) yield loss	References
Lentil	Pod filling	54%	Sehgal et al. (2017)
Groundnut	Reproductive	33%	Carvalho et al. (2017)
Faba bean	Pod filling	58.3%	Migdadi et al. (2016)
Pigeon pea	Flowering	40-55%	Nam et al. (2001)
Soybean	Flowering	24~50%	Oya et al. (2004)
Chickpea	Various	50%	Shah et al. (2020)
Cowpeas	Pod filling	31-44%	Gnankambary et al. (2020)
Green grams	Flowering	20-30%	Sai et al. (2019)
Common beans	Reproductive	60.8%	Daryanto et al. (2015)

Effect of drought stress on initial developmental phases

DS is very complex phenomena; it is very difficult to understand how it affects legume crop performance. The extent of DS effects differ species to species, area to area, soil to soil, duration of DS and agroecosystems. The effects of DS on different growth phases have been described below:

a. Germination

Seed germination is a first step of growth that happens with seed after sowing. Seed germination is a combination of complex processes start from imbibition and complete with embryo growth. So, for start of seed germination the condensed stored insoluble spare substrates present inside the seeds must be hydrated and hydrolyzed to their basic form to supply food to the young embryo. The imbibition is

necessary to re-activate enzymes, which digest the food and generate energy for the growth of young embryo (Bewley and Black, 1994). Seed germination is supposed to be very sensitive to DS (Nadeem et al., 2019). There are three phases of seed germination after sowing i.e., water imbibition, activation of digestive enzymes (Lipase, protease, amylase) and active growth when radicle start to emerge from seed coat. The very first and prerequisite activity after seed sowing is water imbibition (Molina et al., 2018). Thus, water stress reduces germination by limiting water imbibition (Farooq et al., 2009a). Legumes also consist of impermeable hard seed coats compared to the cereal seeds so it takes longer time to absorb free water. However, different crop species, seed type and even varieties may differ substantially in the rate of water imbibition. Crop species and genotypes may show remarkably difference for their initial water demand for germination of seeds, and these differences have been attributed to the several soil water management practices (Bewley and Black, 1994), and soil physical conditions at the time of sowing. It is very hard to describe the effects of low soil moisture contents on germinating seeds by biological terms, however soil water potential gives information on soil water status (Marshall et al., 1996). The quantity of water needed for seed germination is very minute. The inflow of water from soil to seeds depends upon soil water potential, seed types and soil conductivity to seed. Seed water potential is very low as compared to the soil water potential, so the water moves from soil toward seed. Seeds are very vulnerable to abiotic stresses such as limited water supply. DS due to low rainfall or unavailability of soil

water resulted poor seed germination which leads to less plant population or patchy crop stand. With poor seed germination or uneven plant population, so then it is very difficult to explore overall full crop genetic potential. In the arid areas where wetting of the soil and subsequent drying can create hard crust over surface layer of the soil that become a mechanical obstacle and halt seed germination and further causes improper aeration that imposes temperature injury to the young seedlings. So, it is nearly impossible to germinate seed in field condition if the soil moisture is less than required soil moisture.

b. Crop establishment

For any crop, early, healthy and vigorous initial stand leads to higher crop yield (Farooq et al., 2009b). In most of the arid regions, small food grain legumes are facing DS exposure once or more during different growth stages during crop life cycle (Saxena, 1987). DS during the initial vegetative growth stages may halt seed germination and result poor crop establishment (Daryanto et al., 2015). In chickpea, low and patchy crop stand at farmer field increases yield gap compared to the yield trail data at different research stations (Saxena, 1987). DS causes stunted or less growth, retard cell division, cell elongation, reduces crop canopy and expose soil surface directly to the sun light which further causes water losses due to evaporation (Farooq et al., 2009b; Daryanto et al., 2015). Poor crop establishment allow different weeds to grow more efficiently, extract, absorb and utilize higher soil water under limited water supply as compared to the main crop. In areas having arid and semi-arid climate, soil water

contents in the soil may be lower than field capacity. Even after the rainy season, the crops grown on soil conserve moisture, seed germination, and establishment may be affected by the low level of soil moisture contents at the time of sowing. In sub-tropical areas of Asia, high temperatures and high evapotranspiration between the beginning and end of the annual monsoon may result rapid water loss from the soil surface layers. As a result of soil moisture depletion, the soil moisture contents at seeding depth are not enough for seed germination, seedling emergence, and crop establishment. Successful seedling establishment is possible with rapid growth and root development when soil moisture is under optimal level. During the dry period, rapid primary root growth into the deeper layers of soil and the development of lateral roots to extract more water for seedling growth happened (Jordan et al., 1983). The lateral roots could grow through the soil if soil having low moisture contents or even consist of dry layers. Because in dry soil there is more mechanical resistance for root growth and it further increases when the soil moisture level decreases (O'toole and Bland, 1987). Lateral roots are more capable of maintaining root pressure through alterations in root diameter or osmotic adjustment. When the crops are sown at the beginning of a rainy season, the capacity for the quick development of a deep root system is not required initially since the surface soil would be humid at that time. Nonetheless, strong root development and growth are essentials to survive the seedling under the upper soil layer conditions that dried soon. In that case, sufficient water is available in the deeper layers of the soil (Blum, 1996). Having the ability to withstand low

water contents in their leaf tissue and meristem by the plants may play a critical role to protect the seedling and provide contribution for maintaining plant stand. In general, crop seeds need to critical moisture level to realize germination, and this critical level varies for crop species.

Effect of drought stress on crop growth and physiology

DS negatively affects plant physiology and growth by disrupting different physiological and biochemical processes and events such as cell division, cell elongation, photosynthesis, chlorophyll synthesis, nutrient metabolism, ion uptake, and translocation of nutrients and carbohydrates metabolism (Hussain et al., 2018). DS period may be last for few days to months or some time prevail many years. Such drought spell often accompanied by intensive heat which worsening the situation by increasing water evaporation. DS environments is a big hindrance for production of highly profitable crops (Evenson and Gollin, 2003). Legume crops differ in their response or sensitivity to low water stress but what the situation is, overall crop yield is compromised or crop loose its genetic potential (Nadeem et al., 2019). Drought stress (DS) decreases water and mineral uptake, increases root and decreases shoot growth, decreases photosynthesis rate and stomatal conductance (g_s) which reduces carbon dioxide fixation during Calvin cycle (Chowdhury et al., 2016). The reduction of minerals/nutrients uptake or poor stomatal conductance ultimately reduces performance of these parameters leads to reasonable reduction in plant growth (Farooq et al., 2017). Lowering in water availability

impairs seed germination, hamper plant growth because of a reduction in photosynthetic activity, and disturbs the translocation of photo assimilate from source to sink (Chaves et al., 2011). Under low water presence in the soil, the new leaves formation stops, old leaves are fall down and photosynthesis process stops (Karamanos et al., 1982). As a result of DS, a significant reduction occurs in the per unit leaf area (Farooq et al., 2017). Water deficit causes shrinkage of photosynthetic apparatus due to the damage of thylakoid membranes and disturbance of chlorophyll pigment formation in the leaf (Tas and Tas, 2007).

The clear plant response to DS is a lessening in plant biomass, which is the result of a diminished stomatal conductance, prompting leaf reduction and causing a lesser carbon assimilation through photosynthetic process (Bradford and Hsiao, 1982). The effect of water stress relies upon which growth stage plant faced to drought period during the life cycle of the plant and the length of that period (Bradford and Hsiao, 1982). Nonetheless, the most stamped impact regarding the yield loss in legumes ensues when the plants are exposed to DS at the flowering or grain filling stage (Thomas et al., 2004), which can clarify the plentitude of studies during these formative stages. Regardless of whether DS has been the subject of broad exploration from the gene to canopy levels, it actually stays hard to anticipate the impact of water shortfall on plant N status (Gonzalez-Dugo et al., 2010).

Plant growth is influenced by the rate of cell division, expansion, and differentiation. Cell development is one of the most sensitive periods

for drought because of the decrease in turgor pressure (Rezayian et al., 2018). Plants must respond to maintain turgor during drought, which results in a halt or slowdown of cell expansion and cell division, reducing cell size average (Weijde et al., 2017), reduced stomatal conductance, resulting in a drop in carbon uptake via photosynthesis (Christophe et al., 2011), and poor plant development.

Effect of drought stress on nutrient availability

Plant nutrient relationships can also be affected by DS. For example, a recent examination of data from a number of independent studies (He and Dijkstra, 2014) demonstrated that DS reduces the concentration of nitrogen and phosphorus in plant tissue, and many research studies have shown that DS can decrease nutrient uptake from soil (Sardans and Penuelas, 2012). Decrease in nutrients uptake during DS may occur for several reasons, including the limited nutrient supply through mineralization (Fierer and Schimel, 2002), and by reducing nutrient diffusion and mass flow in the soil (Chapin, 1991). DS could also decrease nutrient uptake by affecting the kinetics of nutrient uptake by roots, but this has been not studied well (Christophe et al., 2011). Diffusion of minerals and water occurs osmotically in roots via both a symplastic and an apoplastic pathway up to the endodermis cells that surrounds phloem and xylem. Reaching the endodermis, ions have to be actively pumped from the symplasm to tracheids of xylem as apoplastic transport is precluded by the Casparian strip (Enstone and Peterson, 2002). NH_4^+ and NO_3^- reduction/assimilation does not take place in the same tissue, NH_4^+ being assimilated in roots, and

sometimes transported to shoots (Schjoerring et al., 2002). NO_3^- reduction and further assimilation occurs in roots and/or shoots depending as example upon the plant species, the amount of available soil NO_3^- . But much less is known concerning legume root development. The root system of legumes is less flourished as compared to cereals (Hamblin and Tennant, 1987). In fact, in legumes, root growth vies for carbon with nodule formation (Voisin et al., 2002). As such, when symbiotic N_2 fixation occurs, root growth is limited due to carbon competition within the plant. Both in field and greenhouse conditions, lower root growth was observed for pea plants grown in conditions of low NO_3^- availability compared to plants fertilized with NO_3^- (Voisin et al., 2010). Moreover, due to climate change, plants will be exposed to higher variability of water availability, including increased intensity and frequency of extreme DS (Pereira et al., 2006). Thus, it seems crucial to interpret the effects of DS on plant N_2 fluxes.

Drought stress effects on reproductive growth and grain filling

Grain filling is influenced by both the transfer of assimilate and current photosynthesis stored prior to flowering (Bonnett and Incoll, 1992). After flowering, the amount of assimilate produced by photosynthesis is determined by the plants ability to exploit the limited water available during grain filling (Passioura, 1994). Along with that, the large-scale of pre-anthesis assimilates may be translocated to the grain. The percentage of grain weight that comes from this source varies greatly between species and DS environment,

and is heavily influenced by drought patterns (Passioura, 1994). Drought episodes during faba bean development resulted in lower seed weight according to Grzesiak et al. (1989). DS after pollination often resulted in pods emptiness because of reduction in movement of photosynthate to the newly developing seeds. Similarly, the activities of different assimilate partitioning enzymes badly affected during grain filling stage (Farooq et al., 2009b).

How to manage drought stress in drought prone areas

Since areas vary in their extent to the DS. Presently, the economically feasible ways to help growing crops under water limited conditions are still limited (Li et al., 2000). The strategies to minimize DS may be differ from one area to another. Varieties also may differ to respond DS. Since there are variations in the season of growth and agro-ecological conditions of growing area, crops species have different adaptive mechanism to drought at multiple levels of organization, covering physiology, morphology and anatomy. Different strategies have been used to control these adoptive mechanisms.

Agronomic strategies used to reduce drought stress

Agronomic approaches such as soil tillage, sowing date, soil mulching, cropping pattern, intercropping and varietal selection are most attractive option used to tackle DS.

a. Conservation tillage

Conservation tillage and planting system that covers about 1/3rd or more of the upper soil surface with crop residues, after crop sowing, it could capture more moisture and enhance water availability to crops. Chickpea can be planted early following rice harvest if it is planted under zero tillage conditions on residual soil moisture. (Bimbraw, 2016). Faba bean when sown following the first autumn rains in Mediterranean climate, it avoids drought and produce up to 4.2 t ha⁻¹ seed yields, depending on the seasonal rainfall (Loss et al., 1997). Plant ecologists agree that larger faba bean grain yield is a result of greater initial plant vigor. As a result, more vigor and growth lead to higher seed weight and higher yield (Turk and Tawaha, 2002). In some areas there is hard pan or soil is so compact that reduces water infiltration into the deeper layer of soil. In case of soil compaction subsoiling every 3 to 5 years to break the hardpan and make soil loose to allow water infiltration. In case of heavy soil, natural compaction of soil occur so deep ploughing must be done every 2-3 year.

b. Crop sowing dates & varietal selection

Early planting legumes crop can better cope to overcome DS by early completion of life cycle. Sowing date must be adjusted according to rain forecast to avoid DS. Varieties have also some specific characteristics to perform well if sow on particular dates. Selection of those crops and their varieties which are more suitable to that area and well adapted to the limited water supply are imperative to well cope

with DS (Singh et al., 2014). In drought-prone areas, short duration crops and their cultivars that mature early and demand less water need to be encouraged. To increase profit and water use efficiency, low-water-demanding crops can be diversified with high-water-demanding crops (Bobojonov et al., 2013). Similarly, Zhang et al. (2019) reported that better performance of alfalfa variety was due to high water retention, high osmoregulation capacity and photosynthetic activity, higher antioxidant enzyme activities and lowest lipid peroxidation. Therefore, the crops and their varieties should be preferred based on the availability of water. Chickpea, pigeonpea, and groundnut landraces that develop in their natural environments are frequently affected by terminal DS. Some cowpea genotypes survive under terminal dryness by blossoming 12 days early on an average, while others can go weeks without water and only flower when favorable weather conditions return (Fatokun et al., 2012).

c. Intercropping

Under rainfed conditions intercropping systems should be used to achieve higher, more consistent yields (Singh et al. 2014). In rainfed areas, intercropping in specific ratios can reduce the chance of total yield failure while also improving water utilization (Gautam and Bana, 2014). Intercropping increases crop output and yield per unit of water supplied (Sharma et al., 2017), improves soil water conservation, decreases runoff and increases water supply (Chen et al., 2018), and provides increases in water use efficiency (Raza et al., 2021). Intercropping minimizes evaporation from inter-row, regulates

extreme transpiration, promotes storing of water in root zone, and generates a conductive microclimate for plant growth and development (Zhang et al., 2012). Caihong et al. (2015) reported wheat intercropping with faba bean, Roy et al. (2015) reported corn intercropping with mung bean, Sun et al. (2018) reported corn intercropping with alfalfa produced higher yield and WUE under intercropping as compared to sole cropping.

d. Soil surface mulching

When compared to no mulch or no soil surface cover, straw widely used as mulch conserves more water through the soil profile in the early growth stage of the plants. Following the release of conserved soil water, regulate proper plant hydration status, maintain the temperature of the soil, and provide soil mechanical resistance, resulting in improved root growth and seed yield of both chickpea and mustard were grown in soil covered with straw mulch than that in soil without mulch (Rathore et al., 1998). Conservation agriculture, according to recent studies, increased crop output by 20-120 percent and water productivity by 10-20 percent (Patil et al., 2016). In croplands, the evapotranspiration process is the main source of water loss from soil and plant surfaces (Morison et al., 2008). Unproductive water loss is defined as 30-60% of total applied water that is not immediately utilized by crops (i.e., evaporation). For this reason, lowering unproductive soil evaporation in agricultural areas is a possible strategy to boost water efficiency. Evaporation losses are higher in bare soil which is directly exposed to heat and wind.

Mulching might help to enhance 10-20 percent water use efficiency (Kazemia and Safaria, 2018). Mulching improves the efficiency of water use by increasing the rate of infiltration (Ahmad et al., 2015) and lowering losses of evaporation (Ramakrishna et al., 2006), reduces surface runoff (Ahmad et al., 2015), and changes in soil temperature (Ranjan et al., 2017). Under water stress, Ahmad et al. (2015) found that mulch treatment increased efficiency of water use, weed population, and relative water content of leaves were less, compared to no mulch.

Morphological traits involve in drought tolerance

Growth is a joint adventure of cell division, cell growth, cell elongation, and differentiation. It is not the result of single process but combination of physiological, biochemical, genetic, ecological processes and their interactions. DS has an impact on all of these processes. Because it changes turgor pressure (Taiz and Zeiger, 2006) and involves a complicated relationship between source and sink, cell growth is the most vulnerable to DS. DS inhibits mitosis, cell elongation and cell expansion, and ultimately reduced growth (Hussain et al., 2008). Water deficiency, whether permanent or temporary, has a greater impact on plant growth and development than other climate conditions. Drought has two main effects: reduced germination and the development of weak plants (Kaya et al., 2006). The bean resists water stress by reducing stem elongation and leaf size along with the reduction of number of leaves to reduce water loss (Karamanos and Travlos, 2012). The bean is heavily branched, with

runners running throughout the ground, and produces a large number of leaves and biomass when well-watered (Mitchell et al., 2005). To access subsoil moisture, the bean also possesses a tap root that allows it to reach far beneath the surface (Comas et al., 2013). Water can linger in the root zone for months after rain since the bean grows in sandy soils, and a tap root can access this water. One of the common examples of drought avoidance and adaptation mechanisms is deep root growth (Chandler and Bartels, 2008). In water uptake pathway when liquid-phase water transport via the soil-plant-atmosphere continuum, roots are frequently the site with the most resistance (Kramer and Boyer, 1995). As a result, a critical element in influencing the efficiency of transpiration rate and various drought adaptation mechanisms is soil water uptake by the root system. Water intake by the root is a complicated process that is influenced by root anatomy, structure, and its overall contributions to water transport. (Cruz et al., 1992). With the increase in adaptation of dry pea chickpea, and lentil in semiarid regions of the Canadian prairies, crop production has diversified significantly.

Physiological mechanisms involve in increasing drought tolerance

Evolution of crop plants by a variety of natural processes helps the crop to cope with the DS patterns. Thus, landraces have developed a variety of physiological, morphological, and phenological strategies to efficiently utilize available environmental resources in order to maximize production if irrigation is provided during the reproductive period. This shows that, despite their selection and evolution in certain

conditions, the time it takes these landraces to reach maturity is closely related to stored soil moisture available (Singh and Reddy, 1988).

Although the function of osmoadaptation in cowpea has been debated, rapid and large increases in proline promoting osmotic adjustment have been found in several cultivars under water stress (Costa et al., 2011). Proline accumulated slowly or emerged many days after watering was stopped in other cultivars (Shui et al., 2013). Because of these differences, metabolic changes in cowpea during a water shortage have been attributed to a stress-induced starving injury rather than a positive response. Water losses from the leaf surface by evaporation can be reduced by wax deposits or a thick cuticle on the surface of the leaf (Jefferson et al., 1989). In moisture-limited situations, genotypes with lower residual transpiration rates offer a functional benefit because they utilize water more efficiently (Paje et al., 1988). In soybean and cowpea (Paje et al., 1988), genotypic heterogeneity in residual transpiration has been documented (Walker and Miller, 1986).

Changes in leaf angle are a vital approach enabling legumes to respond to DS. When there is no water shortage, leaflets orient perpendicular to incident light, but parallel to it when there is deficiency of water (Squire, 1990). When dissipation as latent heat is not available due to lack of water, the change in the angle of a leaf can effectively minimize the radiation load on water-stressed leaves, limiting heat damage (Forseth and Teramura, 1986). Reversibility,

rapid recovery from water deficiency relief and little yield decline during water deficit are all advantages of the ability to modify leaf angle or orientation (Shackel and Hall, 1983). When evaporative demand is low and WUE is high, the leaf orientation allows for maximum radiation interception (Muchow, 1985). Almost all legumes respond to radiation and water scarcity with para heliotropism; however, the degree of movement varies between legumes and genotypes within a particular bean species (Ludlow and Björkman, 1984). Some legumes, such as peanuts (Matthews et al., 1988) and bean, have genotypic variability in paraheliotropism (Sato and Gotoh, 1979).

Leaf Surface Characteristics

Water losses from the surface of a smooth leaf are higher when compared with the crinkled leaf that can produce little still air pockets (Rosenberg, 1978). Increased waxiness and pubescence in various plants, especially legumes, increases leaf reflectivity and lowers water loss when they are stressed (Ehleringer, 1980). The transpiration efficiency of soybean lines with dense pubescence is higher (Baldocchi et al., 1985). In water-limited situations, leaf pubescence may have an adaptive advantage since the higher carbon fixation are allowed by hairs, lethal high temperatures are avoided, and water loss is reduced, allowing the plant to extend its growth for a longer duration DS (Ehleringer, 1980). Protect the leaf from direct sunlight, heat, and photoinhibition in beans (*Phaseolus vulgaris* L.), leaf movement or leaf orientation or paraheliotropism). Turgor pressure

fluctuations at the pulvinus at the base of each lamina allow for leaf movement (Pastenes et al., 2005). Light and leaf water status are the two most important factors that influence leaf mobility.

Role of osmotic adjustment or protective solutes in drought tolerance

Osmotic adjustment (OA) is a mechanism in response to drought stress in many plants. During OA, accumulations of different solutes occur as a result of drought stress, and it reduces the osmotic potential of the cell to maintain cell turgor pressure and stomatal conductance (Blum, 2017). The OA is measured by estimating proline, soluble sugars, amino nitrogen, and potassium contents in plants leaf or roots. The accumulation of organic solutes depends upon the extent of stress. Earlier researcher relates OA to the salinity tolerance in plants (Harris et al., 1924). Morgan et al. (1991) reported that genotypes of same plant species differ in their cellular OA potential at different levels of hydration. Firstly, Kemble and Macpherson (1954) reported after extensive research that proline synthesise in plants under drought stress, play a role in OA and also involve in cellular protection (Shabala and Shabala, 2011). Although, OA is resulted from slow and reduce growth but still photosynthesis continue to synthesize sugars and different compatible solutes. In legumes, Morgan et al. (1991) reported that indeed growth is inhibited by drought stress but OA maintain to increase 20% yield under water deficit conditions. OA does not happen suddenly if plant becomes dehydrated rapidly, the adoptive process and accumulation of organic solutes are required

(Blum, 2017). OA is related to the high relative water contents and in chickpea it improves yield up to 60% relative to drought sensitive or unable to adjust their cellular solute concentration (Moinuddin and Imas, 2007). In pea, Sánchez et al. (1998), reported that yield and harvest index were related to the OA or turgor maintenance. OA is highly related to the grain yield of Pigeon pea (Subbarao et al., 2000). Sloane et al. (1990), found that soybean having better OA performed well under drought stress conditions. In conclusion overall legume genotypes having high ability to OA performed better under drought stress conditions.

Stomatal Conductance and Cuticular Characteristics

Stomatal conductance is a measurement of stomatal opening and it can be used to determine the internal water status and exchange of gases in a plant. When water stress happens rapidly it reduces stomatal conductance and carbon dioxide fixation is limited that resulting less photosynthesis (Yathisha et al., 2020). After re-irrigation, these light-driven mechanisms will normally reset by absorbing light energy via chlorophyll-protein complex interactions. Baath et al. (2020) studied the stomatal conductance in three legume crops (moth bean, guar and tepary bean) and reported that stomatal conductance was the main limitation to photosynthesis. On the other hand, a precise balance between lowered stomatal conductance and photosynthetic rate, is more important. Reduced water loss may not be reasonable if it is accompanied with a decrease in net photosynthesis. Among these legumes tepary bean better tolerated against water stress compared to

the mothbean or guar because of its high stomatal closure and high-water use efficiency, combined with vigorous growth during initial phases of growth. As a result, tepary bean grew at the fastest rate and produced the most aboveground biomass even at low water levels. By altering stomatal apertures, stomata attain the best level between the requirement for carbon dioxide and water conservation (Berninger and Hari, 1993). Leaf mesophyll conductance of carbon dioxide has been proposed to be significance as DS rises and carbon dioxide move from intercellular spaces to the stroma of chloroplast (Flexas and Medrano, 2002). Stomatal opening and closing play a major role in regulating water movement during transpiration from the leaf surface so as to meet the evapotranspiration requirement according to the root water absorption capacity; however, this happen only when the fraction of transpirable water reach to 0.3 MPa or below (Sinclair and Ludlow, 1986). This is required to keep internal plant water levels higher than the threshold level, hence aiding dehydration prevention strategies (Turner et al., 1984). Differences in genetics, characteristics of stomata, such as stomatal aperture size, sensitivity to adjust the external and internal water status and density, have been playing a role in maintaining and keeping water status in the plant (Markhart, 1985). Stomatal characteristics are heritable and transfer from one generation to the next generation.

Breeding and biotechnical approaches used to increase drought tolerance

To develop drought resistance in crop plant is a complex phenomenon which depends upon several traits and their interactions like morphological, rooting systems, physiological and biochemical process (Mitra, 2001). The recognition of genes which control physiological, morphological traits and their action is very important for breeding point of view. There are some genetic management options can be used to reduce the effects of drought during reproductive phase of growth. One of the options is selection of early maturing varieties that ripening before the commencement of drought period. This is also known as drought escape. For instance, newly developed short-duration groundnut varieties are considered highly acceptable as compared to traditional long duration varieties (Virmani and Singh, 1986). There are many evident that short-duration legume varieties give higher more stable yields than longer duration ones (Sabaghpour et al., 2006). The first and most important stage in starting a genetic modification program focused at boosting yield and its stability in drought-prone regions is to characterize the drought environment of the target producing environment (Campbell and Diaz, 1988). The development of molecular markers and specific DNA sequences has made it possible to describe the genetic basis of agronomic traits (Thomson et al., 2009). Studies on the genome-wide association applications for complicated traits can made possible for scientist to get useful information for the genetic improvement of food

grain legumes (Nawaz et al., 2020). The development of varieties which give high genetic potential under optimum condition and also produce good yield under DS conditions are highly acceptable. The incorporation of physiological and morphological mechanisms of drought resistance in high yielding but drought sensitive genotypes is very complex because the bases of adaptation of DS tolerant has been not fully understood. So, in contrast, improving the yield potential in already available varieties is more promising by finding genetic variability in genotypes. In below table some useful traits are given while screening or breeding of genotypes.

Table: Drought Tolerant Traits in Different Legume Species Use For Breeding or Screening

Legume species	Drought tolerant traits	Reference
Cowpea	<ul style="list-style-type: none"> • delayed leaf senescence • stem greenness, • deep rooting 	Muchero et al. (2008)
Chickpea	<ul style="list-style-type: none"> • high root depth and biomass, • lower canopy conductance • low transpiration rate • smaller leaf area 	Anbessa and Bejiga (2002). Zaman-Allah et al. (2011)
Soybean	<ul style="list-style-type: none"> • slow canopy wilting • osmotic adjustment (OA) • fibrous and moderate lateral roots • leaf pubescence • ABA, ROS scavenging 	Manavalan et al. (2009)
Broadbean	<ul style="list-style-type: none"> • Leaf thickness • Chlorophyll contents • Root shoot ratio • Leaf temperature • Stomatal conductance 	Link et al. (2007), Siddiqui et al. (2015)
Faba Bean	<ul style="list-style-type: none"> • RWC • proline contents • antioxidants 	Siddiqui et al. (2015)
Mungbean	<ul style="list-style-type: none"> • RWC, Reduction in leaf area 	Bangar et al. (2019)

	<ul style="list-style-type: none"> • Reduction in leaf area • Proline contents • Stomata • Shorter plant stature • Yield 	
Lentil	<ul style="list-style-type: none"> • harvest index • seedling vigour, • biological yield • early flowering and maturity, • high SPAD value, 	Kumar et al. (2012)
Lupin	<ul style="list-style-type: none"> • ability to accumulate assimilates • root–shoot ratios • leaf area 	Palta et al. (2012); Annicchiarico et al. (2018)

CONCLUSION

As per the regular scientific assessments on climate by the Intergovernmental Panel on Climate Change (IPCC) warn about climate change and it also suggests some crop risk management strategies under changing climatic scenarios for sustainable crop production. With the changing climate, more severity in drought or flood may occur in future. Low rainfall or unequal distribution of rainfall may affect legumes performance by affecting seed germination, stand establishment, vegetative growth and reproductive growth stages. To secure food security or sustainable legume production, different crop management options like changing sowing dates, changing cropping pattern, selection of most suitable genotypes which thrive under limited rainfall or water supply may be use to grow crop successfully. Developing drought tolerant genotypes are another option to tackle with DS. In marginal areas where legumes are grown, some soil management practices, manage soil fertility levels, adding

some nutrients should be used to overcome the DS. For breeding purpose some drought tolerant traits like leaf size, cuticle layer, presence of pubescence, root structure and presence of different compatible solutes in the plants should be incorporated into high yield drought sensitive varieties. Checking the weather forecast beforehand is also best option to sketch the crop sowing plan.

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CHAPTER 5

EFFECT, TOLERANCE MECHANISMS AND MANAGEMENT OF SALT STRESS IN GRAIN LEGUMES

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INTRODUCTION

Salinity is the other major abiotic stress factor, affecting 950 million ha of agricultural land in the World (Tepe and Aydemir, 2015). Salinity is effective especially in arid/semi-arid regions of the world. This significant problem is expected to become a more complicated problem in the coming decades (Islam et al., 2013). Salt-affected soils are increasing every year throughout the world (Patil et al., 2015). The salinity problem is increasing especially in South Asia (India, Pakistan) and Australia (Vadez et al., 2007). Both water soil salinity is a serious crop yield reducer also across the West Asia and North Africa countries (Pouresmael and Valiani, 2011). Salinity results in poor yields globally (Qurashi and Sabri, 2013). Improved genotypes adapted to saline soils are needed in these zones (Vadez et al., 2007).

Sodium chloride is the major salt that contributes to soil salinity. The response of crops to sodium chloride excess is highly complex, with changes in plant morphology, metabolism and physiology (Dawood et al., 2014). Soil salinity decreases crop yields by double-action (ionic toxicity and water deficit) on plants (Köse, 2012). It is severely affecting the productivity of salt-sensitive species. Understanding this complex abiotic stress and the physiological and genetic basis of salt tolerance will help to improve the yield of crop cultivars (Vadez et al., 2012). High ethylene concentration under salinity is a major factor for the early senescence of plant parts. Increased levels of ethylene inhibit plant growth and physiology which deteriorate the quality of the pulses (Ahmad et al., 2013). In response to salinity stress, pulses

accumulate proline. Salinity disrupts the physiology of plants, but phytohormones help for regulating the response of plants to salinity stress (Farhangi-Abriz and Torabian, 2018). Auxin is also helping to control salinity by regulating the stress response in plants (Abdel Latef et al., 2021).

Pulses are more sensitive to salinity stresses than cereals (Hobson et al., 2004). Poor seedling germination and crop establishment are the main problems under salinity stress (Keshtiban et al., 2014). Roots of cool-season pulses rarely penetrate one-meter depth. The total root length of pulses is approximately 10 times smaller than cereals. Their root length density rarely exceeds 1 cm root / cm³ soil in the upper or lower layers of soils (Gregory, 1988). Root apex-root apical meristem activity is critical for the modulation of root architecture under salt stress. Pulses have a basic type of open root meristem (Khandal et al., 2017). Salt stress causes toxic effects, nutritional effects and loss of ability for water absorption of plants. The reaction of plants to salt stress is morphological changes (Arefian et al., 2014a). A reason for the reduction of yields of pulses under saline soils is damaged nitrogen-fixation in nodules (Rao et al., 2002). Salt stress disturbs the symbiotic performance of crops and increases plants' susceptibility to soil-borne pathogens (Egamberdieva et al., 2017). Legumes are highly susceptible to soil salinity due to the sensitivity of nitrogen-fixing nodules (especially root hair infection and nodulation). Arbuscular mycorrhiza fungi inoculation is a method to improve rhizobial symbiosis and crop growth under these conditions (Garg and Singla,

2016). Biofilm and exopolysaccharide production are strategies of salt-tolerant bacteria to help the metabolism combat salinity stress (Qurashi and Sabri, 2012). Arbuscular mycorrhiza supports the host plant to fight with negative effects of salt stress (Garg et al., 2014).

Oxidative stress is a major biochemical result of salt stress (Arefian et al., 2018). Salinity ends with cell death at the post-oxidative stress stage (Arefian et al., 2014b). In the plant's mitochondria, alternative electron transport pathways, which is consisting of the alternative oxidase and dehydrogenase families are thought to have a role in the control of oxidative stress response activities in cells (Sweetman et al., 2020). Salinity has effects on plant physiological processes. Yield and productivity reduction under salt stress is frequently associated with the decline in photosynthesis. PSII photochemistry (Fv/Fm) efficiency decreases under salt stress (Ganjeali et al., 2017).

SALT STRESS IN GRAIN LEGUMES

Lentil

Lentil (*Lens culinaris* Medik) is a major pulse in West Asia, North Africa and other regions of the World (Turk et al., 2003). It is an annual cool-season legume crop (Muehlbauer et al., 2006). This species has considerable importance as food, fodder and feed (Oweis et al., 2004). Lentil is a nutritious food for millions of people. They are not only a good source of energy but also a range of prebiotic carbohydrates (Migliozzi et al., 2015). Lentil is rich in folates and

carotenoids (Thavarajah et al., 2017). It is a quick-cooking and protein-rich crop with high iron and zinc (Podder et al., 2020).

Abiotic stresses like drought, salinity, heat, nutrient deficiency, nutrient toxicity and cold stress adversely affect lentil yields worldwide (Al-Quraan and Al-Omari, 2017). Lentil is salinity sensitive and salt stress is seriously affecting crop growth and yields (Kayednezami et al., 2012). Observations of germination of seeds and early growth is a suitable strategy to detect salinity tolerance of lentil genotypes (Foti et al., 2019).



Fig 1. Lentils under salinity conditions (Anonymous, 2018)

Root plasticity helps plants to tolerate salinity in different genotypes of lentil. Various morphological root parameters were tested in lentil seedlings in a genotype considered salt-tolerant (Ustica) and in a salt-sensitive one (Eston), grown on salinized soil in a study. Apart from the root biomass production, two ecotypes showed contrasting root

morphological responses and these might be partially responsible for dissimilar abilities to tolerate salinity (Panuccio et al., 2011).

In order to investigate the salt tolerance mechanism in Egyptian lentils, five cultivars were grown under different salinity levels in another study. The results indicated that Giza 9 is salt-tolerant, while Giza 4 is a salt-sensitive lentil cultivar, as Giza 9 showed the highest germination percentage and relative water content, the lowest decline in root and shoot length, and the highest proline content. It was also found in this study that, ascorbate–glutathione cycle is related to salt tolerance in Egyptian lentil cultivars (Gaafar and Seyam, 2018).

Salt stress inhibited nitrogenase, glutamine synthetase and NADH-dependent glutamate synthase activities. However, nitrogenase activity in nodules is more sensitive to salt stress than glutamine synthetase and NADH-dependent glutamate synthase activities ($\text{NH}_4 +$ assimilation) (Rai and Singh, 1999).

In lentil, ionic toxicity is mainly responsible for salt-induced damages. Ionic toxicity, but not osmotic stress caused chlorophyll degradation. Lentil is very susceptible to K^+ leakage under NaCl stress. Reduction of K^+ leakage is necessary for salt tolerance in lentil. The addition of Ca along with NaCl showed no chlorosis and improved K^+ content. Results demonstrate that prevention of Na-induced K depletion in root might enhance salt tolerance in lentil (Hossain et al., 2017). Seed germination is the most important sensitive stage to this stress, so that early seedling growth and final crop yield can be inhibited by effects

of salinity (Shohani et al., 2014). Metabolomics analysis showed distinctive tolerance mechanisms in discrete plant organs of lentil upon salinity stress (Skliros et al., 2018).

Chickpea

Chickpea (*Cicer arietinum*) is a commonly grown pulse worldwide (Kohli et al., 2014). Its high protein content, dietary fibre, essential minerals contents, the ability for biological nitrogen fixation and adoption to low-input crop production systems make chickpea valuable (Kaashyap et al., 2017). Drought, high-salinity, cold and other abiotic stresses greatly affect chickpea production (Mantri, 2007). Especially it is seriously affected by terminal drought and salinity stresses (Varshney et al., 2009). Soil salinity is a major and increasing problem in chickpea cultivation zones in the world (Samineni et al., 2011). Because, chickpea is the main pulse cultivated often on saline lands in arid/semi-arid zones in the world (Lavrenko et al., 2019). Chickpea plants often experience drought, heat and salinity stresses in combination at reproductive stages (Pushpavalli et al., 2020). This crop is highly susceptible to both soil or water salinity (Boukraa et al., 2013).

Salt stress produces ionic, osmotic and secondary stresses which impair many metabolic processes in plant tissues and results in a decline in crop yield (Buttar et al., 2021). Disturbed redox homeostasis equilibrium for production and removal of reactive oxygen species damages membranes, denatured proteins and

peroxidize lipid, which alters the normal metabolism of crops (Bharti and Garg, 2019). The reproductive phase is more salt-sensitive (Colmer and Vadez, 2014; Khan et al., 2017).

Cultural conditions like soil properties, climate and irrigation methods and other environmental conditions also affect yields of chickpea crops irrigated with salty water or cropped on salt-affected soils also on various and (Rameshwaran et al., 2016). Excess salt in topsoil or subsoil affects crop production. Tolerance of wheat, barley, canola and chickpea to subsoil salinity was compared on a heavy texture soil (sodic vertosol) in a study by Grewal (2010). They harvested plants 40 days after sowing and determined that increasing levels of subsoil salinity (NaCl) depressed shoot and root biomass, root/shoot ratio, K/Na ratio in leaves and Ca/Na ratio in leaves, water uptake and water use efficiency significantly for tested four species. The level of the effect varied among different species. Chickpea was most affected followed by wheat, barley and canola. Their reduction in shoot dry weight of chickpea was 64% followed by wheat (49%), barley (37%) and canola (34%) respectively by highest salinity dose. Water uptake declined by 61% for chickpea, 36% for wheat, 31% for barley and 26% for canola.

Chickpea species are considered salt-sensitive but genetic variation exists for salt tolerance (Turner et al., 2013). Cultivated chickpea has a narrow genetic base which is forcing breeders to produce new elite cultivars with resistance to major abiotic stresses (Mantri et al., 2007). Breeding for salt tolerance is hard to accomplish due to limited

knowledge on key traits changing the crop performance under excess salt. Also, high-throughput phenotyping of large and diverse germplasm collections is difficult (Atieno et al., 2017).

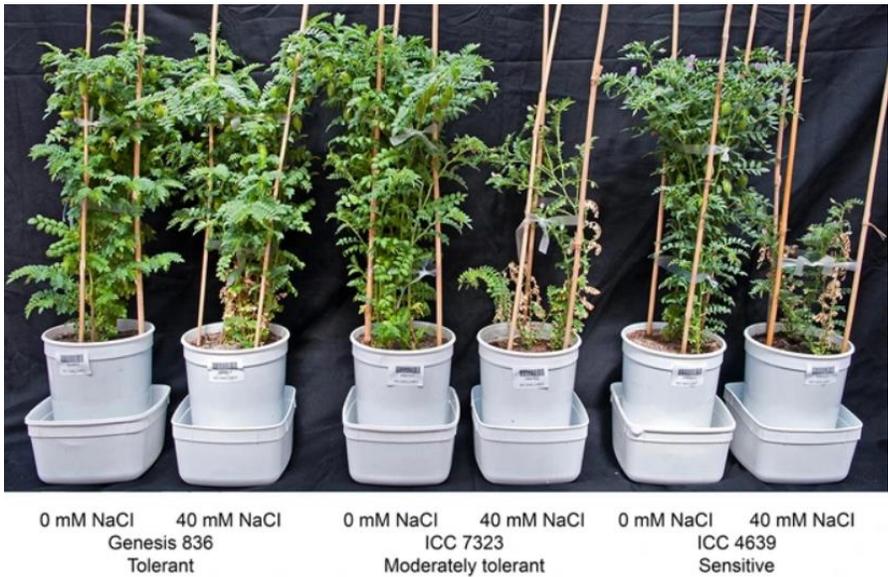


Fig 2. Genotypic variation for salinity tolerance in the chickpea (Atieno et al., 2017)

Soil rhizobacteria with plant growth-promoting help to improve the growth of crops and reduce salt stress effects by regulating multiple biochemical, physiological and molecular processes (El-Esawi et al., 2019). *Sinorhizobium medicae*, *Mesorhizobium mediterraneum* and *Mesorhizobium ciceri* strains showed differing symbiotic performances after inoculation to chickpea under unstressed or salt-stressed conditions in a study by Mhadhbi et al. (2004).

Beans

The common bean (*Phaseolus vulgaris*) is the most important legume for human consumption worldwide (Hernández-Lucero et al., 2014). Common bean is a major pulse also in developing countries but is seriously affected by salinity stress (Hiz et al., 2014). In a study by Khadri et al. (2007), sodium exclusion in the shoot was found as a strategy of common bean to limit Na toxicity under salinity conditions.

Phaseolus vulgaris plants were grown under NaCl stress by Howladar (2014). Plants were sprayed twice with moringa leaf extract at 21 and 28 days after sowing. Moringa leaf extract alleviated the adverse effects of NaCl and applied bean seedlings grew strong under salinity stress. Moringa leaf extract supported the antioxidant system in plants and helped to tolerate environmental stress.

Presoaking bean seeds with propolis extract or maize grain extract were tested by Semida and Rady (2014) to reduce the adverse effects of NaCl-stress. Soaking seeds in either extract, increased the germination percentage of seeds, growth of seedlings, relative water content, total free amino acids, the stability index of cell membrane, free proline concentrations, total soluble sugars, indole-3-acetic acid and gibberellic acid and antioxidant system activity. propolis extract or maize grain extract have supported the tolerance of crops to salinity.

Peas

Field pea (*Pisum sativum* L.) is an important leguminous of the World which is originated from the Mediterranean part of Southern Europe and Western Asia (Wolde and Adamu, 2018). Field pea seeds contain a significant amount of proteins, carbohydrates, amino acids, vitamin A, vitamin C, calcium and phosphorous. It is used for diversified purposes (Jovicic et al., 2010). Pea is essential for human nutrition. Salinity impedes pea growth and yield by damaging the ionic balance, osmotic balance and regulation of hormones (Sapre et al., 2021).



Fig 3. Poor germination of peas and weeds in wet saline soil (Anonymous, 2015)

Salinity is a limiting factor for the productivity of pulses under field conditions worldwide. Field pea is highly sensitive to salt stress which adversely affects germination, length of root, number of leaves, plant

height, leaf area and total dry mass at the vegetative stage of plants (Singh et al., 2013).

Salt tolerance trait is very important required to overcome plant productivity reductions (Sanan-Mishra et al., 2005).

Nine pea genotypes (*Pisum sativum*), were studied under salt stress by Shahid et al., (2012). Salinity stress reduced internode length, fresh biomass, dry biomass, number of leaves stomatal conductance, transpiration rate, photosynthetic rate, chlorophyll content, cell membrane stability index. It increased superoxide dismutase antioxidant enzyme, peroxidase antioxidant enzyme, catalase antioxidant enzyme, proline organic solute, glycine betaine organic solute and total free amino acids, lipid peroxidation, hydrogen peroxide and leaf abscisic acid in plants. Tolerant genotypes produced maximum dry matter, contained a high concentration of glycine betaine, proline and amino acids and high antioxidant activity under saline conditions. Salt tolerance of pea was associated with osmolyte and antioxidant enzyme concentrations in tissues.

Faba bean

Faba bean (*Vicia faba* L.) is used both for human and animal nutrition (Oufdou et al., 2014). Faba bean is important in the Mediterranean region, West Asia, China and Australia and is adapted to neutral & alkaline soil conditions. It is mostly grown under rainfed conditions on saline-sodic soils where yields are limited by salt stress (Tavakkoli et al., 2012). Salinity severely limits faba bean production. Seed

germination of faba bean is sensitive to salinity. Cropping improved varieties exhibiting salt tolerance during the seed germination stage is a good strategy for the cultivation of this crop (Yang et al., 2020).

Faba bean production in semi-arid and coastal zones may be problematic due to the salinity sensitivity of symbiosis of faba bean (Cordovilla et al., 1994).

Effects of seawater on nodules, chlorophylls, proline content and proteins of faba bean were investigated in the study of Fahmi et al. (2011). Plants were irrigated with water mixed with seawater at 20, 25, 30, 40 and 50% concentrations. Plants were inoculated with eight Rhizobial isolates. Nodules number, nitrogenase activity, nitrogen content and chlorophyll a and chlorophyll b content were decreased with increased salinity with all isolates but proline accumulation was increased. ARC2 isolate was best with the highest parameter values; except for proline accumulation.

Auxin controls development processes and regulates stress responses in plants. Abdel Latef et al. (2021) investigated the role of exogenous indole-3-acetic acid (IAA) in salt tolerance in the *Vicia faba*. Foliar application of 200 ppm IAA under 60 mM and 150 mM salt stress promoted root and stem traits. IAA application reduced proline and increased soluble protein, soluble sugar and total free amino acid contents of stem, roots and seeds. IAA also increased nodule number under salinity stress. IAA improved mineral homeostasis (Ca^{2+} , K^{+} and Mg^{2+}) and inhibited the excess accumulation of Na in roots. As a

result, exogenous IAA application increased the yield of faba beans under salinity conditions.

Cowpea

Salinity is a major abiotic stress factor affecting the yield of cowpea in arid / semi-arid regions (Gogile et al., 2013a). In their study, Gogile et al. (2013b) showed the intraspecific variation in cowpea (*Vigna unguiculata* L.) cultivars for salt stress related to early biomass accumulation. Salt tolerant cowpea varieties differ in protein profiles and use different strategies to overcome salinity stress. Salinity-tolerant varieties show induction of protein function for photosynthesis and energy metabolism.

Consumption of salty irrigation waters in cultivated areas increasing and preventing optimal growth and development of cowpea. Low rainfall in semi-arid/arid zones prevents the leaching of salts from soils. The seedling stage is highly vulnerable to cowpea growth and development. Very few salt-tolerant cowpea cultivars were reported globally (Dong et al., 2019).

The seedling stage is the most salt-sensitive stage in cowpea. A rapidly screening methodology for a large number of genotypes will help cowpea breeding for salinity tolerance (Ravelombola et al., 2019).

CONCLUSIONS

Pulses are sensitive to salinity in soil or irrigation water. Growing tolerant species like barley, canola, etc may be a better strategy on saline soils. But salt-tolerant genotypes exist and screening global variety collections for salt tolerance may help to find more varieties tolerant to salt. Arbuscular mycorrhiza may be applied to salt-affected soils to help host plants resist the negative effects of salinity. Need global researches to isolate salt-tolerant N fixing bacteria from rhizospheres of pulses globally. Need global researches to find an economic and easy method to significantly reduce the salt content of irrigation waters.

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CHAPTER 6

EFFECTS, TOLERANS MECHANISMS AND MANAGEMENT OF COLD/CHILLING IN GRAIN LEGUMES

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INTRODUCTION

Low temperature is an important environmental factor affecting the distribution, growth and yield of plants on earth. Recently, 6% of all usable agricultural soil in the world is exposed to cold effect. It is predicted that the world population, which increases by an average of 90 million every year, will reach 8.5 billion in 2050. For this reason, it is thought that the shrinkage of agriculturally workable areas and agricultural production because of biotic and abiotic stresses with the increase in population in the world can cause great harm, especially when viewed from the perspective of human nutrition. Therefore; It was necessary to open these cold areas where agricultural activities do not take place in order to carry out agricultural applications, to examine the cold resistance of field crops that have high nutritional content in their structures, and to grow cold-resistant plants. In addition, the understanding of the physiological and biochemical mechanisms that supply resistance to cold in plants and help adaptation is of great importance in terms of expanding the cultivation of agriculturally produced products and growing plants that have the ability to withstand (Turan, 2007).

According to the terminology related to cold stress; temperatures between 0 and 12⁰C are defined as cold stress, and temperatures below 0 °C without snow cover are defined as frost stress (Toker et al., 2007).

Developing chilly resistance is important for chilly climate legumes and their wild relatives (Toker, 2005; Toker et al., 2007). Because chilly

resistance is accepted as the basic component of winter cultivation (Bond et al., 1994; Arbaoui and Link, 2008). When plants are exposed to low temperatures, a lot vicissitudes consist at the physical, biochemical and molecular grade.

Cold stress is an important abiotic condition affecting legume production. Low temperature affects physiological mechanisms like gas barter, soil humidity content, formation and uptake of nutrients. Thus, it creates a direct and indirect effect on the unlike functioning and characteristics of plants and causes an effect on their periods in the ecosystem (Gornall et al., 2010). Most legumes are submitted to cold stress pending the flowering and seed filling phases, leading to damage of quality and revenues of crops. The harmful impact of heat stress primarily be attached to the time of exposure to heat with the development phase of the plant. For instance, winter legumes such as field peas, lentils, broad beans and chickpeas are tolerant to fallen temperature. However, they are best exposed to heat stress during the vegetative period (Bita and Gerats, 2013). Chilly stress, which adds both chill damage (less than 20 °C) and frost damage (less than 0 °C), is one of mainly momentous limiting elements in vegetative and generative stages of plants (Lang et al., 2005). Low heat results in reduced leaf area and less dry matter in winter legumes. Chlorosis is common in plants that have undergone cold shock. In later periods, necrosis due to chlorosis occurs in old leaves (Gogoi et al., 2018a).

Cold stress affects plant growth, crop productivity. It is also an environmental stress factor that limits product quality, post-harvest life,

and geographic distribution of economically important species. The effects of cold stress on Grain Legumes are as follows:

1. EFFECTS OF COLD STRESS AT THE CELLULAR LEVEL

Temperature is an environmental stressor that changes seasonally and undergoes daily fluctuations. In order to survive in extreme high and low temperatures, plants develop two different mechanisms: escaping from stress conditions or resisting stress conditions. However, extremely low temperatures cause ice formation in plants and cause irreversible damage (Browse and Xin, 2001). The mechanism of avoiding stress is to decrease the influence of stress. Plants escape from stress by changing their morphological structure (leaf surface and thickness, size and density of stomata, chemical composition and thickness of the cuticle, size and chemical composition of roots and stems). In the case of resistance to stress conditions, the effects of stress are repaired or removed (Browse and Xin, 2001).

The most important changes in cold adaptation; growth reduction or cessation, decrease in water content in tissues, a temporary increase in ABA level, changes in cell membrane fat composition, saving of osmotic regulatory material (proline, betaine, polyol and sugars) and increase in antioxidant level (Xin and Browse, 2000). These low molecular weight substances help the plant to survive by accumulating in high concentrations without harming the cell metabolism (Williamson et al., 2002). There are many studies showing that osmolytes such as proline, stress hormones, fatty acids and sugars

increase in various plants during cold stress (Savitch et al., 2000; Nayyar et al., 2005a; Arbaoui and Link, 2008).

Many changes occur in lipid combination of cell membrane during cold acclimation. Along coldness adaptation, there is an increase in the phospholipid ratio of the cell membrane first. This enhancement in the phospholipid rate was mostly watched in many plant species like oats, rye, Jerusalem artichoke, arabidopsis, and pigweed. However, the degree of this increase varies according to the plant species. Secondly, when frost resistance reaches its maximum level after the cold adaptation stage, the proportion of cerebrosides in the cell membrane decreases (Uemura and Steponkus, 1999). In many plants, frost resistance can be increased by gradual exposure of the plant to non-freezing low temperatures, known as cold adaptation, or by external application of ABA (Bakht et al., 2006).

When sensitive plant tissues are exposed to cold stress, the cell membrane changes from the liquid phase to the solid phase. So, cell membrane permeability and activation energy of cell membrane enzymes increase. Continuing stress with the accumulation of toxic substances and imbalances in metabolism leads to cell and tissue damage or death. With the short-term cold stress or the return to normal temperatures, the plant returns to its normal metabolic conditions. Chloroplasts are the first and most severely affected organelle in cold stress. Prolongation of the stress period leads to the breakdown of chloroplasts. Mitochondria, nuclei and other organelles are less susceptible to cold damage (Kratsch and Wise, 2000).

Cold harmony; It causes accumulation of cold protective substances (sugar, proline, etc.), changes in enzyme activity, physical and biochemical changes in membrane structure and components. Reactive oxygen species in high concentrations can damage cell organelles. This leads to cellular fragmentation. Plants to prevent or mitigate these damages; they developed enzymatic antioxidant systems like superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase and peroxidase with natural antioxidants (Prasad, 1997); Scebba et al., 1998; Scebba et al., 1999; Lee and Lee, 2000; Kuk et al., 2003; Huang and Guo, 2005; Posmyk et al., 2005).

The cold resistance of the plant depends on the balance between carbohydrate metabolism at low temperatures. Plants accumulate intracellular water-soluble carbohydrates (sucrose, glucose, fructose, raffinose, stachiose, fructoses, and oligosaccharides) as a protective response to low temperatures. It is known that sugars directly modify the cell membrane (Klimov et al., 2002).

Also, when plants are exposed to low temperatures, plant shoots turn red and accumulate anthocyanins in vacuoles. Increasing anthocyanin accumulation with decreasing temperature has been associated with cold resistance (Leng et al., 2000).

2. COLD DAMAGE OCCURRING IN THE VEGETATIVE PERIOD

Every single plant has ideal temperature requirements suitable for its expanding and recovery. Temperature requirements that are optimal for

one plant can cause stress for other plant. Native to warm habitats, most plants show symptoms of cold damage when subject to fallen, non-freezing temperatures. Especially crops such as corn, soybeans, cotton, tomatoes and bananas are sensitive to temperatures below 10-15 °C and symptoms of cold damage occur. Stress symptoms that cause damage to these plants seem between 48-72 hours. Yet, this process varies for each plant and depends on the plant's sensitivity to cold stress (Mahajan and Tuteja, 2005).

Various phenotypic indications like decreased leaf enlargement, fade, chlorosis, tissue and cell death may develop in response to cold stress (Mahajan and Tuteja, 2005). Heat stress affects all developmental stages of plants. Low soil temperatures significantly affect the emergence and early seedling development periods. Germination time is prolonged, emergence rate decreases and the plant remains smaller (Prasad et al., 2006).

Soil temperatures do not fluctuate as fast as air temperatures. However, in many species, exposure to low temperatures decreases root growth and root hydraulic conductance, and food uptake can be inhibited. Generally, underground organs are more sensitive to cold than aboveground organs (Fennell and Markhart, 1998; Domisch et al., 2002). Roots and leaves have different sensitivities to cold stress. This difference is associated with different respiratory responses to cold. While total respiration decreases in leaves, total respiration increases in roots (Hu et al., 2006). Generally, it has been watched that the seeds that do not meet the need for rest are slow to germinate and develop

early seedlings at low temperatures. It has been observed that the cold adaptability of young seedlings is better in annual species grown in cold environments than plants in flowering stage or mature stage (Bois et al., 2006).

Cold stress during germination in chickpeas increases susceptibility to soil-borne diseases. As a result, the number of seeds is reduced. In addition, since it causes seedling death, it directly causes low yield (Croser et al., 2003). Similarly, in soybeans 1 °C (Posmyk et al., 2005); in peas 3 °C (Badaruddin and Meyer, 2001); in broad bean 5 °C for 24 hours (Hamada, 2001); in chickpea vegetative damage occurred early in the period, less than 10 °C from pod start to ripeness (Kaur et al., 2008); -10 °C for 15 and 30 minutes (Heidarvand et al., 2011) while completely seedling killing occurred below excessive chilly (Badaruddin and Meyer, 2001).

3. COLD DAMAGE IN THE GENERATIVE CIRCUIT

Frost damage usually occurs when the daily minimum temperature remains below 0 °C. Flowering, the beginning of the pod setting and the seed filling period are the periods when legumes are most susceptible to frost damage. Mechanical damage to the tissue occurs due to ice formation. Frost sensitivity also varies among legume species. The low temperature causes the intracellular water to turn into ice and consequently the cells to shrink, which in the end leads to withering and plants death (Stoddard et al., 2006). Unsuitable temperatures at generative phase disrupt flower formation, fertilization

and the gametophyte stage. Later, the fertilized flowers are poured, fruit formation is reduced and low grain filling leads to yield losses (Hedhly et al., 2009; Thakur et al., 2010).

Frost damage critical heats for chickpeas appear to be higher than for peas, lentils and broad beans. Accumulating substances dissolved in cells confer tolerance to the nature of any of the abiotic stresses. Of the legumes, the pigeonpea is the most susceptible to frost damage at any stage of growth. The first place where frost damage affects Pigeonpea is the thermal apical tip. Apical branches damaged by frost turn brown and die. Since most of the flowers in the plant are shed, the pod development and grain filling process stops abruptly. Therefore, the generative period in the plant is equally sensitive to frost. Chickpea is a more obscure species than other legumes. It will continue to produce new flowers and pods if the temperature and soil moisture are suitable, except during frost periods when there is a negative effect. This allows chickpeas to compensate for the early flower loss caused by frost or cold damage. In a controlled-condition pot study with two bean and lentil genotypes, the plants were exposed to sub-zero temperatures. At study, it was come to a decision that the beans and lentil genotype showed tolerance to cold in the tests (Stoddard et al., 2006).

Chickpeas, lentils and peas from winter legumes are particularly responsive to falling heat in the time of pod creation and seed charging (Maqbool et al., 2010). Falling heats affect the carbohydrate metabolism and lead to gametophyte sterility as the energy required for the reproductive organs cannot be provided (Nayyar et al., 2005b;

Oliver et al., 2005). Clarke et al. (2004) stated that development of pollen tube is not complete if the chickpea is not fertilized enough, and that there is no pod formation in case such plants experience cold stress. Kumar et al. (2010), reported in their work that lower temperatures reduce the growth of the pollen tube, thus leading to failure in fertilizing.

Board and Kahlon (2011) reported that the damage to soybeans exposed to cold throughout flowering and pod-sealing periods significantly reduces yield compared to those exposed close to the ripening period. Kurosaki and Yumoto (2003) found that there was more damage in cold-susceptible genotypes. It has been reported that the saving of storage proteins, minerals and amino acids is prevented by the decrease in grain filling, grain filling time and speed, depending on the relationship between the decreasing nutrient and amount in chickpea under cold conditions (Nayyar et al., 2007).

Shafiq et al. (2012), reported severe chilly damage along flowering and pod-fixing periods in their study with pea varieties brought from different countries. Low temperature damage to peas was more severe on buds, flowers and pods. This caused the resulting particles to be smaller.

Even an increase of 1-2 °C above the threshold temperatures at which plants grow critically affects the harvest and harvest-interested qualities of legume plants (Kumar et al., 2013; Kaur et al., 2008). Low heats during day and night in chickpeas cause cold stress. Because of this,

spills occur in the flowers (Nayyar, 2005a). Peas (*Pisum sativum* L.) are also not tolerant to freezing temperatures (Stoddard et al., 2006).

4. EFFECTS ON PHOTOSYNTHESIS AND RESPIRATION

Plants exposed to cold in the light and in the dark have different reactions to cold. Photochemical inhibition in the dark and at low temperature rarely occurs. In contrast, the combination of low temperature with light leads to sustained photochemical inhibition. The decrease in temperature in the light decreases the reaction rates of especially CO₂ fixation and photorespiration, which generally occur in photosynthesis (Allen and Ort, 2001)

Low temperature inhibits the light reactions of photosynthesis. In plants exposed to low temperatures for a short time, net photosynthesis is inhibited because of the rallying of soluble sugars. In addition, photosynthetic electron transport may be inhibited in several chilly responsive to plant species (Bertamini et al., 2005). In fact, the physiological effects of many environmental stresses, like falling temperature, are the ability to upset the equalize between the absorption of light energy and the light energy used. This helps reduce O₂ and generate reactive oxygen species (ROS) (Logan et al., 2006). Environmental stress agents like chilly stress in light can increase the formation of harmful oxygen species. The high sensitivity at low temperature is due to restricted carbon metabolism. High concentrations of reactive oxygen species trigger genetically programmed cell death. Along with light, reactive oxygen species have

harmful effects on photosynthetic systems, such as degradation of antioxidants and pigment whitening, which is defined as photooxidation (Foyer and Noctor, 2005).

The rate of photosynthesis, which changes depending on the temperature, varies accordingly the plant varieties (Hikosaka et al., 2006). High light intensity and low temperatures photo-oxidize the photosynthetic machine in chickpea (Nayyar et al., 2005a,b,c) impair electron carrying, disable rubisco and close stomata, resulting in decreased CO₂ digestion. Georgieva and Lichtenthaler (2006), reported that cold stress reduced chlorophyll fluorescence in their study on two pea varieties. It was determined that the rate of photosynthesis decreased by more than 50% in Soybeans subjected to cold stress overnight (Van Heerden and Kruger, 2000; Van Heerden et al., 2003). They reported that cold stress decreased the amount of photosynthesis in soybeans (Board and Kahlon, 2011), beans (Tsonev et al., 2003) and broad beans (Hamada, 2001). Performances of plants exposed to heat stress are directly related to their yield from photosynthesis. Respiration in plants is related to temperature. An increase in respiration in response to cold has been detected in cold-stressed plants (Kaur et al., 2008). Kumar, et al. (2005b) stated that a 68% reduce in cellular respiration occurred in chickpeas at low temperatures (5-13°C) in their study.

The cytochrome pathway through which the available electrons are transported in plants under low temperature is blocked. Plants in this situation use alternative respiratory tracts such as AOX (alternative oxidase) and PUMP (plant uncoupling mitochondrial protein) to

increase respiration rate (Vanlerberghe, 2013; Chocobar-Ponce et al., 2014). Indeed, it has been reported that the increase of AOX protein in mitochondria in mung bean and pea plants under cold stress is a result of the use of alternative transport pathways (Vanlerberghe, 2013). In the study in which different cold stresses were applied to bean seeds, it was reported that while the grains at room heat respiration for 7 days, the seeds at 3 °C for 5 days, and the seeds exposed to -19 °C cold do not respiration due to freezing cold. It was concluded that this situation occurred because the AOX and PUMP pathways were used (Srivastava, 2015). Georgieva and Lichtenthaler (2006), in their study using two *Pisum sativum* cultivars, stated that there was a decrease in photosynthetic activity after exposure to low temperatures, but there was an increased recovery rate in photosynthesis within 48 hours during the subsequent recovery period. The decrease in the rate of photosynthesis during the adaptation of plants to the cold reduces the amount of water in the tissues with the growth of plants. Therefore, dissolved substances accumulate in tissues (Margesin et al., 2007). Carbohydrates accumulate in peas beneath cold conditions (Streb et al., 2003). Additionally, a link was set up among the sugar content in plant leaves and frost resistance (Bourion et al., 2003).

5. EFFECT OF COLD STRESS ON GRAIN DEVELOPMENT

Grains develop depending on the development of legumes in vegetative and generative periods. The dimensions of the grains depends on the cell division, grain refill and maturation duration (Thuzar et al., 2010). Cold stress disrupts the entire reproduction cycle, starting with

pollination. This situation limits grain formation and development (Thakur et al., 2010).

5.1. Synthesis of Starch and Protein

The decrease in pollen germination rate in plants under cold stress is thought to be a result of limited synthesized and accumulated energy reserves (Srinivasan et al., 1999). This may be related to high ABA, which decreases the sugar grades in plants (Nayyar and Kumar, 2005). Since the pollen tube grows poorly, maturing and next seed growing are prevented. It has been determined that such a situation is a possible reason of cold-connected product decline in chickpeas (Clarke et al., 2004).

5.2. Grain Fill Rate and Time

Basically, chilly stress decreases grain filling ratio and time, damages photosynthetic structures in edible grain legumes and causes blockage in accumulation pathways (Gogoi et al., 2018b).

All ecological tension conditions along seed growth have a powerful effect on grain product and qualification (Thakur et al., 2010). Nayyar and Kumar (2005) stated that the low temperature (5/13⁰C average min./max. temperature) along the breeding and seed refill periods in chickpea influences the formation of the grain, the filling speed of the grain, the time, the grain yield and quality. Low temperatures affected all grain filling periods. For example, cold stress during the generative period of chickpea reduced the seed filling ratio

and time (Kaur et al., 2008). When the average daytime and night temperatures (28/17⁰C with 5/13⁰C) to which chickpea were subjected to, were compared, it was determined that average average of grain left and dimension reduced by 41% and 24%, in order of (Nayyar and Kumar, 2005)

In chickpeas under cold stress, it has been reported that as a result of decrease in leaf area, deterioration in metabolism or inhibition of enzymes, the ratio of grain to filling time decreases, pod shedding increases and smaller grains occur (Thakur et al., 2010). Nayyar and Kumar (2005) have detected that chilly stress (5/13⁰C average min/max temperature) increases electrolyte leak, causes membrane harm, decreases chlorophyll concentration and photosynthetic action, and leads to lower photosynthesis products in their study in chickpea.

5.3. Supply of Carbohydrates and Growth of Grain

In the course of seed refill; carbohydrates, proteins and lipids accumulate in growing grain. Much ecological stresses such as aridness, temperature and chilly have been determined to change carbohydrate metabolism in plants (Gogoi et al., 2018b). Thakur et al. (2010) reported changes in the carbohydrate content of plants under low temperatures. Invertase, which is attached to the cell wall, breaks down the sucrose in the area where the phloem is depleted. This has an indispensable act for seed refill in obtaining more sucrose and facilitating the delivery of hexoses to dividing cells and metabolizing degradation products (Thakur et al., 2010). Beneath fallen temperature, cell wall-bound

invertase activity reduces. Thus, as it causes sucrose accumulation, it inhibits cell cleavage in endosperm and weight decrease occurs in the grain Cheng et al., 1996).

Therefore, cold stress, which causes a change in invertase activity, hinders the reproductive development of carbohydrate metabolism and leads to a decrease in yield in legumes (Gogoi et al., 2018b) .

5.4. Hormonal Irregularities During Grain Maturation

Abscisic acid (ABA) and cytokinin, which are important phytohormones, play a significant act in filling phase of grain as they play a role in increasing the biomass of the grain during the development of soybeans (Schussler et al., 1984). Phytohormones expand endosperm cells by mediating cell division during the developmental stages of the grain. Thus, it regulates the speed and direction of the assimilate flow (Hansen and Grossmann, 2000).

Grain filling was affected by changing the accumulation of hormones in chickpeas exposed to low temperature (Devasirvatham et al., 2015). Increased ABA grade in leaves supports the turning off of stomata, reducing the CO₂ levels in the cell. This leads to a decrease in photosynthesis (Devasirvatham et al., 2015; Fischer et al., 1986).

In the growing grain endosperm, higher cytokinin levels needed for cell division have been observed in the early stages of grain filling (Dietrich et al., 1995; Banowetz et al., 1999). Beneath low temperature, the amount of cytokinin in the plant reduces owing to induction of oxidase

enzyme, which slows down the assimilation flow by reducing the rate of cell division. Nayyar (2005b) reported an increase in polyamine levels of chickpeas subjected to cold stress (12-15⁰C/4-6⁰C, day/night) and decreased during prolonged exposure.

6. STRATEGIES FOR INCREASING TOLERANCE OF LEGUMES AGAINST COLD STRESS

6.1 Selection and Breeding

It may be possible to develop cold-tolerant plant genotypes by using molecular, conventional and ohmic-based techniques together (Maqbool et al., 2017). Traditional breeding methods have been effective in obtaining cold resistant varieties in many different crops (Jha et al., 2017).

It is well known that heat stress and escape mechanisms reduce the harmful effects it causes in most legumes. However, the best selection strategy is to identify superior genotypes only under real field conditions (Gogoi et al., 2018b).

Wild species are considered valuable gene sources for replication, particularly in resistance to abiotic stress tolerance (Sharma et al., 2013). However, it is used extremely poorly because it has some obstacles in hybridization studies. Despite this, the use of wild relatives as gene sources according to cultivars remains an important breeding criterion (Gogoi et al., 2018b). In regions where winter chickpea is cultivated, the yield drops significantly due to the fall of flowers at low

temperatures in the spring. It has been reported that two closely related annual wild chickpea species (*C. reticulatum* and *C. echinospermum*) are very resistant to low temperatures. They reported that a number of adapted genes expand the genetic base in these species and that cold-tolerant genes are beneficial (Bajaj et al., 2015).

Photoperiod sensitivity, which causes delayed flowering and maturation in plants with abiotic stress tolerance, increases tolerance to cold stress. Therefore, it is very important to cultivate suitable varieties for the regions (Gwata and Siambi, 2009).

6.1.1. Legume genomes

In recent years, genome sequences have been structured in chickpea (Varshney et al., 2013) and soybean (Schmutz et al., 2010) in studies on legume genomes. In addition, genome sequencing and transcriptome sequencing were also performed in alfalfa, lupine, peanuts and peas (Gogoi et al., 2018b).

6.1.2. Quantitative Feature Loci (QTL) and Marker Assisted Selection (MAS)

Existing genome sequences in many legumes have enabled the identity and use of molecular markers as well as the creation of molecular maps (Varshney et al., 2013). Next generation sequencing (NGS) techniques have found many different applications in transcriptome sequencing (Hiremath et al., 2011), epigenetics (Jackson and Chen, 2010), and gene statement analysis (’t Hoen et al., 2008). In addition, genetic

engineering has guided the process of identifying molecular markers with genome coding using NGS in some legume genomes (Ma et al., 2012).

Quantitative trait loci (QTL) related to seed mass, absorption, germination and heterotrophic growth were detected in the relevant chromosomes (chromosomes 1, 3, 4, 5, 7 and 8). Thus, cold stress molecular research was supported.

For similar purposes, 161 QTL and 679 markers were detected in winter peas. Among them, two sets of QTLs are associated with a gene locus (Hr and Le) concerned with to freeze tolerance in pea. However, it has been determined that grain quality and productivity are independent of each other in frost tolerant winter peas (Klein et al., 2014).

In a study conducted on two cold-sensitive (*Phaseolus angustissimus* L.) and more tolerant (*Phaseolus vulgaris* L) bean species, it was reported that DNA binding factors, a few warmth shock proteins and phytochrome P450s play a very important role (Vijayan et al., 2011).

6.1.3 Functional Genomes and Transgenic Development of Cold Sensitive Genes

a. Transcription Factors and Other Genes

Transcription factors have a very important place in the determination of cold tolerant candidates in the future. In the study conducted in soybean, DREB homologues were identified as GmDREBa,

GmDREBb and GmDREBc. Also, TFs such as WRKY, DREB2A, a DREB homologue of GmWRKY21, and STZ/Zat10 have been upregulated and have reported roles in soybean resistance to cold stress (Zhou et al., 2008). Cheng et al. (2009) found that excessive secretion of GmCHI and GmGT-2A-GmGT-2B controlled by the CamV35S promoter in Arabidopsis increased the cold tolerance of the plant.

Su et al. (2014) reported that TFs such as GmbZIP1, which they isolated from soybean, regulate stomatal closure in plants. In addition, an ICE1-CBF3-COR sequence gene sensitive to cold tolerance has also been identified in cowpea (Tan et al., 2016). Devasirvatham et al., (2015) stated that the high conversion grades of CmMet-2 and CarNAC1, a metallothionein-like gene found in chickpea, in plants under cold stress indicate that the gene is cold tolerant of chickpea.

b. Cold tolerant transgenic edible legumes

Although there are few studies on transgenics in chilly stress-related legumes, this has been resolved in a lot large crops. Genes from legumes were validated in the model plants, some of which are described below:

In an exhaustive study investigating the act of L-asparaginase in conferring low temperature resistance in legumes such as soybeans, it was reported that L-asparaginase released aspartate and NH_4^+ , leading to the identification of GmASP1, which encodes a 326 bp protein that confers cold tolerance (Cho et al., 2007). In similar context, GmMYBJ1 isolated from soybean showed enhanced freeze tolerance when

overexpressed in Arabidopsis (Su et al., 2014). The same gene had previously made Arabidopsis more resistant to cold stress (Sanghera et al., 2011). GsCBRLK, a calcium/calmodulin dependent kinase, showed tolerance to low temperature in transgenic Arabidopsis plants and stated that it plays an important role in the cold stress period (Yang et al., 2010).

Researchers have also shown that CarNAC1 is induced in different parts of chickpea. However, induction has been reported a lot in seeds. They also noted that leaf age is a crucial factor for gene induction, therefore influencing seed germination and development (Peng et al., 2010), supplying a new field for the inclusion of transgenics in this field.

c. mi-RNAs and low temperature stress

Small non-coding RNA molecules called mi-RNAs involved in RNA post-transcriptional regulation have been shown to increase abiotic stress tolerance in plants (Sunkar and Zhu, 2004; Sunkar et al., 2007). Similarly, it has been reported that miR396 increases leaf development (Liu et al., 2008) and its amounts increase up under cold stress (Liu et al., 2008).

7. APPLICATION OF PLANT GROWTH REGULATORS IN COLD STRESS

Hormones regulating plant growth have been extensively worked in plants beneath a wide diversity of abiotic stress situations. They also

have a very serious share in adaptation of plants to abiotic stress conditions. When plants are under abiotic stress, they synthesize many phytohormones depending on the power of their defensive mechanisms (Nadeem et al., 2016). ABA is synthesized in answer to plants under cold stress. This situation contributes to the plants' resistance to stress (Liu et al., 2008). Abscisic acid, Jasmonates and Salicylic acid activate phosphoprotein cascade pathways that lead to statement of genes related to chilly stress resistance in plants (Kolaksazov et al., 2013). It has been reported that Abscisic acid applied to maize increases the cold tolerance (Xin and Li, 1992), while the amount of Abscisic acid increases in varieties that are resistant to cold (Farooq et al., 2009).

In addition to abscisic acid, Salicylic acid is a chemical that is accepted to exist in many different plant species against drought and cold tolerance (Farooq et al., 2009). It has been determined that salicylic acid applied to plants significantly reduces cold (Miura and Tada, 2014) and frost damage (Taşg n et al., 2003).

8. USE OF PLANT NUTRIENT ELEMENTS

Plant Nutrients increase the toleration of plants to stress conditions (Waraich et al., 2012). Nitrogen (N) fertilization of plants alleviates the negative influences of abiotic stresses (Waraich et al., 2011). In the shape of NO, it acts as a scavenging element against ROS and preserves the plant against stress conditions (Wendehenne et al., 2001; Waraich et al., 2012)

It has been determined that calcium applied to red spruce under fallen heat stress increases amino acid, polyamine and chlorophyll contents in the plant (Schaberg et al., 2011). Ca is an necessary food for the fulfillment of stomatal activities in cold-tolerant genotypes (Waraich et al., 2012). It has been stated that Ca in *Vicia faba* positively affects the biological yield of the plant, the plant-water relationship and the amount of chlorophyll, while reducing membrane leakage (Abdel-Basset, 1998). Mg improves root development, improves uptake of water and foods from the roots, and Boron increases the transport of sugar in the plant. It also develops seed germination and seed shape stages (Waraich et al., 2012). Se increases tolerance to cold stress (Hawrylak-Nowak et al., 2010). These studies show that the use of phytonutrients is a potential option to sustainably mitigate the adverse states of cold stress for better plant growth and increased productivity.

d9. SEED COATING

In studies conducted with different plant species, it is thought that seed coating is good for plants against abiotic stress conditions (Hasanuzzaman et al., 2011; Hussain et al., 2016; Wang et al., 2016; Samota et al., 2017). It is an application that allows plants to get used to stress conditions by combining physiological and biochemical stages in growing plants (Hasanuzzaman et al., 2011; Samota et al., 2017). Seed coating with various plant hormones and chemicals can move an significant act in minimizing the inverse impacts that may occur on plants exposed to drought and cold during the first seedling stages. In seed-coated tobacco seeds, application improved the antioxidant

defense system, promoted seed germination and early growth of seedlings (Xu et al., 2011). By coating the seeds with AA, SA and H₂O₂, the plants showed better growth under inappropriate heat (Ahmad et al., 2014; Hussain et al., 2016). Seenium and SA coating of seeds of different rice varieties are effective in increasing cold resistance. They reported that seeds coated with 2.5% Potassium hydrogen phosphate or 2.5% Potassium hydrogen phosphate + Potassium nitrate initiate chill tolerance in the early stages of growth than unprimed seeds (Farooq et al., 2009). Increased chlorophyll and relative water content, antioxidant activity, biological yield and seed yield (Singh and Usha, 2003) The evidence listed above shows that preparing seeds with different treatments presents a realist answer to rice the stress tolerance of plants against unsuitable temperatures.

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CHAPTER 7

**EFFECTS, TOLERANCE MECHANISMS AND
MANAGEMENT OF HEAVY METAL TOXICITY IN GRAIN
LEGUMES**

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INTRODUCTION

Soil has formed the main breeding environment in agricultural activities, especially from the time when mankind moved from hunter-gatherer to settled life. In the future, the soil will not lose its priority as the main growing medium of crop production. Although different cultivation techniques and environments have been developed depending on the advances made in cultivation environments in agricultural production, the soil will not lose its importance.

Stress is defined as a situation that occurs in a plant when environmental conditions change to such an extent that it affects the normal growth and development of a plant negatively (Büyük et al., 2012). Heavy metal pollution, which is an important environmental problem and one of the stress sources in plants, is one of the important problems that agricultural production is exposed to. Heavy metals can be defined as metals with a density of more than 5 g/cm³, an atomic number greater than 20, and causing toxicity and pollution in the environment where they interfere (Apaydın, 2005). The main sources of heavy metals are mineral fertilizers, sewage waste, biocides, wastewater, urban wastes, exhaust gases of motor vehicles and mining (Önder, 2012). Agricultural production is limited in areas exposed to heavy metal pollution. Depending on the level of pollution and the type of pollutant, plant production may not be possible. In this context, soils should be cleaned with one or more of the phytoremediation methods. There are hyperaccumulator plants used for this purpose. Except for a few legumes used for this purpose, the remaining legumes are significantly

affected by the heavy metal level of the environment in which they are grown. However, in recent years, several legume species containing metal-tolerant *Rhizobium* species have been identified in areas contaminated with heavy metals (Fagorzi et al., 2018). Legumes are an important plant species that contains 22% protein, 32% fat and 7% carbohydrates and meets the vegetable protein needs of the world population (Abdelkader et al., 2017). Legumes also can add nitrogen to the soil with the *Rhizobium* species they establish a symbiosis with (Mandal and Bhattacharyya, 2012). Therefore, it is inevitable that soils are contaminated with heavy metals and have a significant effect on the nitrogen fixation of legumes. While the presence of both essential and heavy metal elements such as copper in appropriate concentrations in the soil may increase microbial growth (Meena et al., 2014), even very low values of heavy metals such as cadmium may have a negative effect on nitrogenase activity and therefore on the activities of bacteria (Lebrazi and Fikri- Benbrahim, 2018). On the other hand, in recent years, these symbiotic relationships have been used to clean soils from heavy metals and increase their productivity (Ike et al., 2007; Pajuelo et al. 2008a; Dary et al. 2010; Pajuelo et al. 2011). The benefit of symbiotic relationships here is by promoting plant growth and thus promoting greater uptake of metals (Hao et al., 2014). Data on the heavy metals limit values in soil for some *Rhizobium* species are given in Table 1.

Table 1. Limit values of heavy metals in the soil to which some Rhizobium species can tolerate (Hao et al., 2014)

Species	Cu mM	Zn	Cd	Ni	Pb	Cr	As	Reference
<i>Rhizobium</i> sp	0.5- 2.0	0.5- 2.0	0.1- 1.0	0.25- 1.00	3- 6	-	1- 10	Pajuelo et al. 2008a
<i>Sinorhizobium</i> strains	1.4	-	-	-	-	-	- 10	Pajuelo et al. 2008b; Fan et al. 2011
<i>Mesorhizobium</i> strains	2.2	16- 32	0.3- 0.5	-	-	9.6	-	Wani et al. 2008; Vidal et al. 2009; Hao et al. 2012
<i>Bradyrhizobium</i> strains	1.5	0- 21.5	<0.5	5.1	2	-	2	Wani et al. 2007; Dary et al., 2010
<i>Azorhizobium caulinodans</i>	-	-4-5	-	-	-	-	-	Zheng et al. 2005

1. LEGUMES

The products that make up the pulses group are one of the food groups that have been cultured and produced by people since ancient times, and they are of great importance in human nutrition. Turkey is the gene center of legumes and its ecological conditions are suitable for legume production. For this reason, legumes, one of the traditional agricultural products in Turkey, come second after cereals in field crop cultivation areas (Ceritoglu et al., 2020). As of 2018, 1.224 million tons of production was realized on an area of .8 million decares. Chickpeas, beans and lentils constitute 98.3% of the total legume production. Chickpeas are 630 thousand tons, red lentils are 310 thousand tons, dry beans are 220 thousand tons (TUIK, 2019). Legumes are important foods in terms of protein, dietary fiber, minerals (iron, zinc and magnesium) and vitamins (mainly folate). In addition, many

phytochemicals, saponins and tannins in their structure have a protective effect against cardiovascular diseases and cancer. Their glycemic index is also low (Gül Sarioğlu and Velioglu, 2018).

Legumes widely consumed around the world are kidney beans, white beans (*Phaseolus vulgaris* L.), broad beans (*Vicia faba* L.), chickpeas (*Cicer arietinum* L.), dried or cracked peas (*Pisum sativum* L.), mung bean (*Vigna radiata* L.), cowpea (*Vigna unguiculata* (L.) Walp.) and several varieties of lentils (*Lens culinaris* Medik.). There are also lesser-known varieties such as lupine (eg *Lupinus albus* L, *Lupinus mutabilis* Sweet) and bambara bean (*Vigna subterranea* L.) (FAO, 2016).

Legumes increase soil fertility and reduce dependency on nitrogen fertilizers, due to their biological nitrogen fixation. Environmental and socio-economic hazards that may occur as a result of the use of leguminous plants and the use of nitrogen fertilizers are also minimized (Yavaş and Ünay, 2018).

2. HEAVY METALS

Heavy metal is a metal that has a relatively high density and is toxic or toxic even at low concentrations. In fact, when the definition is classified according to their atomic numbers, metals with atomic numbers greater than 20 or with an amount of more than 5 grams occupying a cubic centimeter of volume are called heavy metals. More than 60 metals in the heavy metal group, mainly lead (Pb), cadmium (Cd), iron (Fe), cobalt (Co), copper (Cu), nickel (Ni), mercury (Hg) and

zinc (Zn) (Kahvecioğlu et al., 2009). Some of these can be micronutrients (Fe, Cu, Zn, Mn, Mo, Ni) for plants and animals and are not toxic unless they exceed a certain limit (Okcu et al., 2009). It is stated that Cd, Pb and Hg are the heavy metals with the most severe toxic effects (Çepel, 1997). The main problem with heavy metals such as Cd, Cu and Hg is that they are not biodegradable and therefore can remain in the soil for a long time. Their presence in the soil may be natural or of anthropogenic origin (Lebrazi and Fikri-Benbrahim, 2018). Melting, metal forging, burning of fossil fuels, etc. Industrial pollution, including industrial pollution, is another source of metal pollution (Khan et al., 2009). In addition, inappropriate waste handling and acid mine drainage are particularly important sources of heavy metal pollution in agricultural areas surrounding mining areas (Williams et al., 2009).

Heavy metals can spread to the environment as fine particles or dissolved (Lombardo et al., 2001). Since heavy metals also pass into the soil, they are transmitted to plants through the soil (Caselles et al. 2002). It is estimated that 22.10^3 Tg of Cd, 939.10^3 Tg of Cu, 783.10^3 Tg of Pb, and 1.35 Tg of Zn are introduced into soils each year (Lebrazi and Fikri-Benbrahim, 2018). If chromium, nickel and lead are found in soils between 10-100 mg/kg and cadmium below 1 mg/kg, these amounts are considered normal levels. While chromium is an essential microelement and is toxic to mammals and other animals at high concentrations, nickel is a possible carcinogenic element for the same group of organisms (Yıldız, 2001). According to Kabata-Pendias

(2010), toxic levels of these metals in shoots reach 10-100 mg kg⁻¹ d.w. for Ni, 1-2 mg kg⁻¹ d.w. for Cr and 30-40 mg kg⁻¹ d.w. for Co. The roots accumulated Ni, Cr and Co at 167.1, 20.5 and 8.5 mg kg⁻¹ d.w., respectively.

3. TOLERANCE MECHANISMS OF GRAIN LEGUMES AGAINST HEAVY METAL STRESS

Legumes try to resist heavy metals by various mechanisms. They first show this with changes in their nodules. The most important change observed in the nodules under toxic metals is the thickening of the cell walls and this can prevent the disruption of symbiosis with metal stress (Sujkowska-Rybkowska and Ważny, 2018). IAA (indole-3-acetic acid), an important phytohormone affecting cell division and differentiation, stimulated plant growth and nodule performance in legume-rhizobia symbiosis. It has been reported that approximately 80% of rhizobacteria can produce varying degrees of IAA (Khalid et al., 2005).

Metal-treated nodules showed structural changes, namely increased accumulation of phenols and higher cellulose loss, wall thickening with hemicellulose, pectins, glycoproteins, and kalose content (Sujkowska-Rybkowska et al., 2020). Lafuente et al. (2015) revealed the induction of synthesis of phenolic compounds (phenylpropanoids, isoflavonoids) in the nodules of *Medicago* plants in the presence of arsenic. The cell wall structure, which includes cellulose microfibrils and non-cellulosic neutral polysaccharides embedded in the pectin matrix with proteins and phenolic compounds, confers metal-binding ability, and excessive

accumulation of toxic metals in the wall often leads to hardening and thickening of the wall (Probst et al., 2009; Krzesłowska, 2011). These glycoproteins are involved in different cellular and developmental processes and response to different biotic and abiotic stresses (Lamport et al., 2006). In root nodules, these glycoproteins in the nodule parenchyma act as intercellular localization, diffusion barriers that help protect them from nitrogenase inactivation (Vanden Bosch et al., 1989; Rathbun et al., 2002). These thickenings consisted of two layers: a dense outer layer containing numerous electron-dense particles of various sizes, including Pb, Zn and C, and a less dense inner layer without electron-dense particles (Krzesłowska, 2011). Another modification of the CW polysaccharide composition under heavy metals stress is the appearance of callose (Hall, 2002). Callose synthesis is induced by various biotic and abiotic stress factors. It acts as a physical barrier against many stress factors that may threaten to penetrate the protoplast, such as biotic threats such as fungal pathogens (Bolwell et al., 2002) or abiotic threats including injury (Jacobs et al., 2003), and heavy metals stress (He'maty et al., 2009).

Another resistance mechanism of legumes against heavy metals is the increase in pectin content. Pectins are found in all cells and make up about 30% of type I primary walls that typically occur in dicotyledons. Secondary walls and type II primary walls, which are most characteristic of grasses, normally have much lower pectin content (Pelloux et al., 2007; Caffall and Mohnen, 2009). The main functional groups of pectin are hydroxyl, carboxyl, amide and methoxyl. These

functional groups can be used to bind heavy metals, especially hydroxyl groups (Kupchik et al., 2006). In particular, an increase in low methylesterified pectins was frequently observed in the walls of metalized roots (Krzesłowska, 2011) and nodules (Sujkowska-Rybkowska and Borucki, 2015). Pectins play an important role in the binding of metals with hemicellulose (Krzesłowska, 2011). Wall thickening may be the result of the hardening of the polysaccharide network. Deesterified pectins can be crosslinked with calcium ions or metal ions, which affects cell wall mechanical properties and metal immobilization (Krzesłowska, 2011).

In the case of heavy metal stress, one of the tolerance mechanisms seen in legumes as in other plants is the changes in phenolic compounds. As a matter of fact, it was reported that the biosynthesis of phenolic compounds was stimulated by the effect of nickel in wheat, aluminum in corn and cadmium in bean plants. It is thought that changes in the metabolism of phenolic compounds occur as a result of hydrolysis of conjugates under heavy metal stress (Sanal, 2019). It is stated that the increase in soluble phenolic compounds is necessary for the formation of a physical barrier that prevents the entry of heavy metals into the cell by increasing the cell wall resistance. As a result of their study, Sujkowska-Rybkowska et al. (2020) stated that the accumulation of phenols and the rearrangement of the nodule apoplast can counteract the harmful effects of Ni, Co and Cr on symbiosis. The protective role of phenols against toxic metals formed by metal chelation and the removal of harmful reactive oxygen species formed in the presence of

toxic ions are well known (Sharma et al., 2012). Lafuente et al. (2015) revealed the induction of synthesis of phenolic compounds (phenylpropanoids, isoflavonoids) in the nodules of *Medicago* plants in the presence of arsenic. Raklami et al. (2019) showed that the gene encoding phenylalanine ammonia lyase was expressed in the nodules of *M. sativa* plants inoculated with metal-resistant strains and grown in the presence of metals. This enzyme is the starting point of two important pathways: secondary metabolism of phenolic compounds and lignin synthesis.

In the face of heavy metal stress, Bacterial exopolymers are generally acidic heteropolysaccharides with EPS-associated ionizable functional groups (e.g. hydroxyl, carboxyl, and phosphoric acid) that exhibit high affinity for certain metal ions (de Oliveira Martins et al., 2008). Microbial metabolism, such as legumes extracellular polymeric substance (EPS) production and enzyme activities, can immobilize and/or alter the redox state of metals to reduce their toxicity to plants. Some *Rhizobium* strains can also adsorb and accumulate metal, which indirectly aids in phytostabilization (Hao et al., 2014). Ion exchange, complexation with negatively charged functional groups, adsorption and precipitation are the mechanisms involved in metal biosorption on EPS (de Oliveira Martins et al., 2008).

One of the tolerance conditions to heavy metals is the increase in the amount of AGP in legumes. AGPs might be transported as a compartment of the endomembrane flow participating in AGP exocytosis to the apoplast in which AGP has a role as pectin plasticizers

(Lampart et al., 2006). AGP decreases the formation of pectate gels and be affecting cell wall extension (Serpe and Nothnagel, 1994). AGPs are formed in the infected cells of root nodules and play a pivotal role in symbiotic nitrogen fixation (Fruhling et al., 2000; Kirova and Kocheva, 2021).

Another mechanism developed in heavy metal stress is the changes in callose content. Generally, callose is a structural component of plasmodesmata (PD) which regulates the intercellular exchange of ions and molecules, including hormones, proteins, peptides and nucleic acids by changing the size exclusion limit (Roberts and Oparka, 2003). High amounts of callose accumulating in the plasmodesma prevent cell-to-cell transport under heavy metal stress. This results in the prevention of a broad invasion of toxic metal ions, however it inhibits the transport of other molecules such as example signaling molecules. In all treated plants, callose accumulation in the CW, particularly in the root elongation and differentiation sites and in the plasmodesmata, is related to inhibition of cell elongation (Sivaguru et al., 2000). Heavy metal stresses callous accumulated in the plasmodesmata. Callose deposition in Plasmodesmata was detected in *A. thaliana* under all stress (Sivaguru et al., 2000) and in *L. minor* exposed to Pb (Samardakiewicz et al., 1996). Heavy metal treatment also promoted nodule callose accumulation in the plasmodesmata between infected and uninfected cells. Callose regulates permeability of plasmodesmata and restricts the movement of metal ions in the cytoplasm (O'Lexy et al., 2018).

4. RESULT

Grain legumes, which have an important place in human nutrition, try to show resistance against heavy metals in the environment where they are grown by various mechanisms. When heavy metals accumulate excessively in plant tissues, they cause changes in various growth processes related to vitality (Phalsson, 1989). Examples of these are mineral nutrition (Costa et al., 1994), transpiration (Lidon et al., 1993), photosynthesis (Nussbaum et al., 1988), enzyme activity (Doncheva et al., 1996), nucleic acid structure (Somashekaraiyah et al., 1992) chlorophyll biosynthesis (Munzuroğlu and Geçkil, 2002) and germination (Ouzounidou et al., 1992) causes changes in plant vitality events (Ouzounidou et al., 1992). In addition to these, physiological events such as damage to membranes (Kennedy and Gonsalves, 1987), disruption of hormone balance, and change in water relationship can be added. In addition, trace metals, especially non-essential ones, can displace the appropriate ions to form their molecules or bind to sulfhydryl and carboxyl groups, thereby disrupting enzyme functioning and thus changing their conformation (Hall, 2002).

The toxicity of heavy metals on legume-rhizobia symbiosis and symbiotic nitrogen fixation varied according to legume and rhizome species, soil and metal species, degree of contamination, as well as nodulation and plant growth promoting activities of rhizome under metal stress (Oves et al. 2010). Legumes especially try to tolerate or resist heavy metal stress with changes in the amounts of stem cell wall

components (cellulose, hemicellulose, pectins, EXT, AGP and callose) (Sujkowska-Rybkowska et al., 2020).

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CHAPTER 8

THE RELATIONS BETWEEN SOIL REACTION AND LEGUME CULTIVATION

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INTRODUCTION

Legumes are among the most important cultivated plants worldwide. Legume cultivation improves soil fertility and is generally used to improve net productivity, increase the soil carbon sequestration (Spehn et al., 2002; De Deyn et al., 2011; Li et al., 2010). Legumes, with their capacity to fix atmospheric nitrogen have a potential to improve soil organic matter and N status (Nichols et al., 2001). Therefore, legume plants generally are used as a cover crop or composted manure in agricultural systems (Snapp et al., 2005).

Legumes can form new root organs known as nodules, and they maintain a symbiotic life with rhizobia in these organs. As a result of this symbiotic life, nitrogen fixation occurs. Nodule formation can be limited by numerous factors such as; soil reaction, as well as effectively regulated by the plant. Most common legume species can be grown in soils having low pH. However, low pH conditions inhibit nodule development (Brett et al., 2013). Soil reaction restricts legume cultivation by the effects on plant, bacterium and nodule formation and function. It affects N₂ amount being fixed and total N content of agro-ecosystem (Rice et al., 1977). It was known that continuous legume cultivation acidifies soil by the release of protons from roots (Yan et al., 1996). Williams (1980) reported that soil pH reduced by almost one unit due to continuous clover production for more than 30 years in Australia.

Effective nodulation is necessary to optimum development and production of legumes planted in soils with low nitrogen content. However, soil conditions affecting rhizobium and host plant are very complex to nodule formation processes (Burns and Norton, 2018a). Generally, insufficient nutrient cycle in the soil, acidifying effects of nitrogen fertilizers and soil leaching cause low soil pH (Martikainen and De Boer, 1993; Von Uexküll and Mutert, 1995). Low soil pH is responsible for 50% or more yield loss as well as legumes such as lentils, beans and peas in wheat and barley (Bordeleau and Prévost, 1994, Vassileva et al., 1997). Interestingly, legumes tend to acidify the soil more than most other plant species (McLay et al., 1997. Tang et al., 1998).

In this section, the relationships between soil reaction and legume cultivation are reviewed including role of soil reaction in plant breeding, factors affecting soil reaction, soil reaction under legume cultivation, nodulation and nitrogen fixation related with soil reaction.

1. Effect of Soil Reaction on Plant Nutrition and Soil Quality

Soil reaction represents soil acidity or alkalinity and is declared as soil pH. The pH is one of the most important soil properties for plant production. The soil pH measures the active hydrogen ions (H^+) in soil solution. Mathematically, the pH scale is logarithmic and inversely indicates the active hydrogen ion concentration in soil solution. A soil having a pH, which is 7 or greater, is called as neutral or alkaline. If the soil has pH less than 7, it is called as acidic. Soil reaction is an

important factor having effects on crop yields, plant available nutrients, and microorganism activities (Anonymous, 2021).

Soil reaction affects the solubility, mobility, and bioavailability of nutrients, which determine their translocation in plants (Förstner, 1995). The mobility reactions of nutrients between solid and liquid soil phases depend on soil pH (Förstner, 1995; Rieuwerts et al., 1998) with respect to pH dependent charges in organic and mineral soil fractions. The soil reaction affects on solubility of elements in soil solution. The dissolution and adsorption of minerals depend on the acidic functional groups in soil. The deficiencies of the basic cations (Ca, Mg, and K), and phosphorus are commonly related with acidic soil condition. Micro nutrients (Fe, Mn, Zn, Cu and Al) cause toxicity to plants in acidic soils (Figure 1). On the other hand, alkaline soils are also related with deficiencies of micro elements and phosphorus (Lindsay, 1979). Micronutrients are generally soluble because of high desorption and low adsorption at acidic soil condition. Adsorption of micro nutrients changes within a narrow pH range from almost non to complete adsorption (Bradl, 2004)

The increasing the level of soil acidity is important nutrients availability like phosphorus, they may become less available to plants (Figure 1). Some heavy metals, like micro nutrients and aluminum, become more available and sometimes toxic to the plants with reducing crop production (Anonymous, 2021).

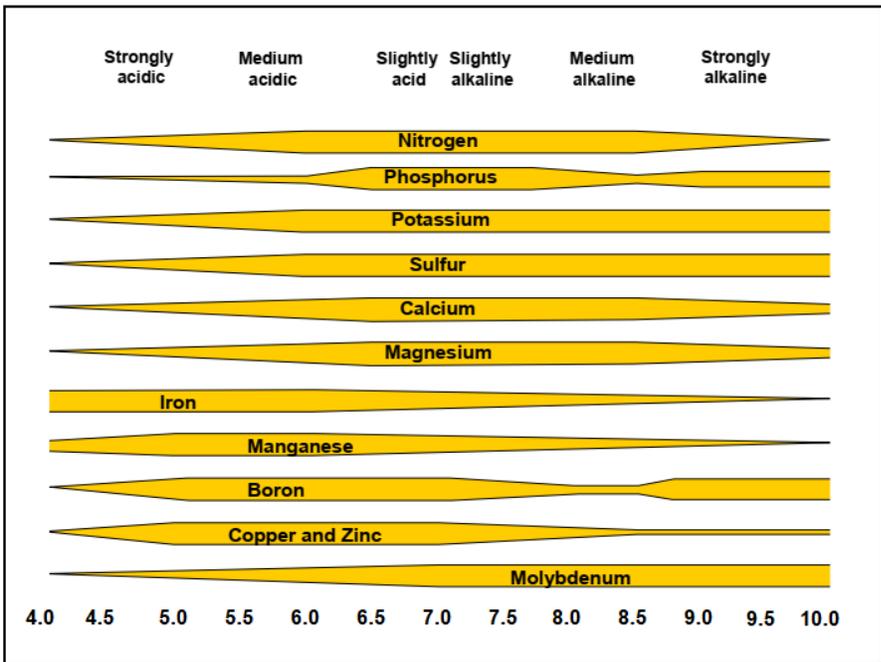


Figure 1. The relations between in soil pH and availability of plant nutrients.

Andersson et al. (2000) reported that the solubility of organic fractions in soil is controlled by soil pH, and increases with dissociation of acid functional groups. Soil pH controls the solubility of organic matter with influencing the charge density of the humic compounds and either increasing the microbial activity. It was reported that required soil pH values are in range of 5.5–8.8 for microbial activity (Fierer and Jackson, 2006, Pietri and Brookes, 2008a,b). In acidic soil conditions, fungal activity is generally higher than bacterial activity because of fungi are more adapted to low pH conditions than bacteria (Anderson, 2003).

Extracellular enzymes produced by soil microorganisms cause biogeochemical cycling of nutrients in soil (Turner, 2010). Proper soil pH level is important for regulating enzyme activity in soil (Pawar, 2015; Sinsabaugh, 2008), and may indirectly effects on enzymes with affecting microorganisms (Keeler et al., 2008).

Soil quality is defined as the capacity of a soil function which sustains plant and animal productivity, maintains the quality of water and air and supports human health and habitation (Karlen et al., 1997). Soil degradation causes high soil strength, low infiltration, high surface runoff and erosion. To protect soil against degradation and erosion, legume cover crops are used successfully to improve soil physical, chemical and biological properties. Blanchart et al. (2004) found that legume ley or pasture roots have a great restoration of physical properties, increase in organic matter content and carbon rhizodeposition, and soil faunal activity of a degraded vertisol soil. Gülser (2004) compared some physical and chemical soil quality indicators influenced by different legume crop species (Alfalfa, Crownvetch, Subterranean clover) in a clay soil. He found that legume cropping treatments improved soil chemical quality over the bare soil with significantly increasing *exch. K* contents from 1.46 me/100 g to 1.55 me/100 g, organic matter (OM) content from 2.28% to 2.67%, total N from 0.19% to 0.26%, electrical conductivity (EC) from 0.31 mmhos/cm to 0.70 mmhos/cm and significantly decreasing soil pH from 6.54 to 6.16 (Table 1). Also, he reported that soil physical quality improved by the legume cropping over the bare soil with

significantly increasing structural stability index (SSI) from 57.4 % to 62.64%, total porosity (F) from 45.28% to 51.57%, volumetric water content (Θ) from 18.77% to 26.00%, infiltration ratio (I) from 0.56 cm/h to 4.89 cm/h and significantly decreasing soil bulk density (BD) from 1.45 g/cm³ to 1.28 g/cm³, penetration resistance (PR) from 2.94 MPa to 1.99 MPa. He concluded that especially alfalfa can be integrated in cropping systems to improve soil quality and for sustainable soil management.

Table 1. Effect of different legume species on some soil quality indicators (Gülser, 2004).

Crop species	pH 1:1	EC (mm h cm ⁻¹)	OM (%)	N (%)	Ca	Mg	K
					(me 100 g ⁻¹)		
AL	6.16c	0.70a	2.67b	0.26a	31.54	8.74c	1.55b
SC	6.21bc	0.52b	2.51c	0.21b	34.41	10.43ab	1.49bc
CV	6.15c	0.51b	2.35d	0.20b	33.10	9.68b	1.50bc
Bare soil	6.54a	0.31e	2.28d	0.19b	34.91	10.16ab	1.46c
LSD	0.13**	0.01**	0.15**	0.02**	-	0.89**	0.08**

Crop species	SSI (%)	BD (g cm ⁻³)	F (%)	θ (%)	I (cm h ⁻¹)	PR (MPa)
AL	62.64a	1.31b	50.57a	26.00ab	2.92b	2.29cd
SC	61.84a	1.28b	51.57a	23.30abc	4.89a	1.99cd
CV	62.52a	1.30b	51.06a	22.22bcd	2.45b	2.44abc
Bare soil	57.44b	1.45a	45.28b	18.77d	0.56c	2.94a
LSD	2.75**	0.09*	4.78*	4.00**	0.86**	0.51**

2. Factors Affecting Soil pH

Soil pH is affected by some inherent factors such as; climate, mineral content and soil texture which are not changed easily. The natural soil pH is also affected by the combination effects of the soil forming factors such as; parent material, time, topography, climate, and organisms. In dry climates, soil pH is generally neutral or alkaline due

to less soil weathering and leaching. In acidic soil conditions, liming application to reach optimum pH helps to increase availability of some essential nutrients, calcium and magnesium, and also increase microbial activity, and improves soil structure.

The pH is one of the most important soil properties affecting soil productivity and strongly influences cultivated plant variety and type of used fertilizers. Soil reaction can be regulated to improve soil quality by some agricultural practices such as; liming, choosing suitable fertilizer and crop species. The vegetation, land use and management have effect on soil pH. It is known that soils under forestland vegetation tend to be lower pH than grassland area soils. Drastic pH changes occur on lands converted from forest or grassland to cultivated land in a short terms.

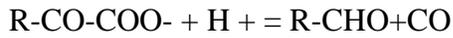
The changes in soil pH are caused due to loss of organic matter, removal of elements uptaken by crops, loss of the soil surface by erosion, and effects of fertilizers. Generally, using nitrogen and sulfur fertilizers can leads decreasing of soil pH level over time.

3. The Changes in Soil Reaction Under Legume Cultivation

The benefits of legumes on soil quality can be summarized with increasing organic matter content of soil, improving soil structure and porosity, recycling plant available nutrients, decreasing soil pH level, increasing microbial activity in soil, and controlling of plant disease and weed problems (Anonymous,1998).

Inoculated, nodulated legumes have the effect of lowering the pH of the soil because they take their nitrogen from the soil as diatomic N, not nitrate from the air. Alfalfa and soybean cultivation lowered the soil pH level in a clay loam by one pH unit. Legumes generally reduces soil pH and provide a suitable growing environment for plants and microorganisms in soils (Anonymous, 1998).

The cultivation of legumes lowers soil pH level by the proton release from roots. As a result of proton release, plants accumulate organic anions decomposed in the soil and neutralize the soil acidity (Yan et al., 1996). Hauter and Steffens (1985) reported that legumes symbiotically supplied NH_3 , do not take up nitrate ions. Nitrate when taken up by plant cells, recycles H^+ , transferred out by the plasmalemma ATPase, back into the cytosol with the effect that increasing pH level in the adjacent soil. When the plants are not fed with nitrate, the activity of the plasmalemma H^+ pump acidifies soils. This H^+ supply is the principal ion pump in plant tissues bringing about a separation of charge and resulting in a pH difference, protons accumulating in the rhizosphere are equivalent to the OH^- primarily produced by the H^+ pump (Schubert et al., 1990; Serrano, 1989). Smith and Raven (1979) reported that hydroxyls retained in the cytosol stimulate the carboxylation of phosphoenol pyruvate; it leads to the production of organic anions. The principal representative of organic anions in most plant species is malate. Organic anions, not organic acids, provide a pH increase when decomposed in soil by the given reaction (Yan et al., 1996).



Yan et al. (1996) determined that cultivation of field beans for 45 days caused a significant reduce in soil pH from 6.00 to 5.64 (Table 2, Figure 2). They also reported that returning field bean shoots increased soil pH from 5.64 to 6.29 significantly, and soil acidification occurred by legume cultivation can be remediated by incorporating crop residues into soil.

Table 2. Soil pH change by field bean cultivation and return of field bean shoots into soil at original soil (pH_0), at harvest (pH_1), at 18 hours after incorporation (pH_2) and at the end of 307 days incubation (pH_3) (Yan et al., 1996)

Treatment	pH_0	pH_1	pH_2	pH_3
+Shoots	6.00	5.64(± 0.05)	6.29(± 0.02)	5.86(± 0.01)
-Shoots	6.00	5.64(± 0.05)	5.73(± 0.01)	5.84(± 0.01)

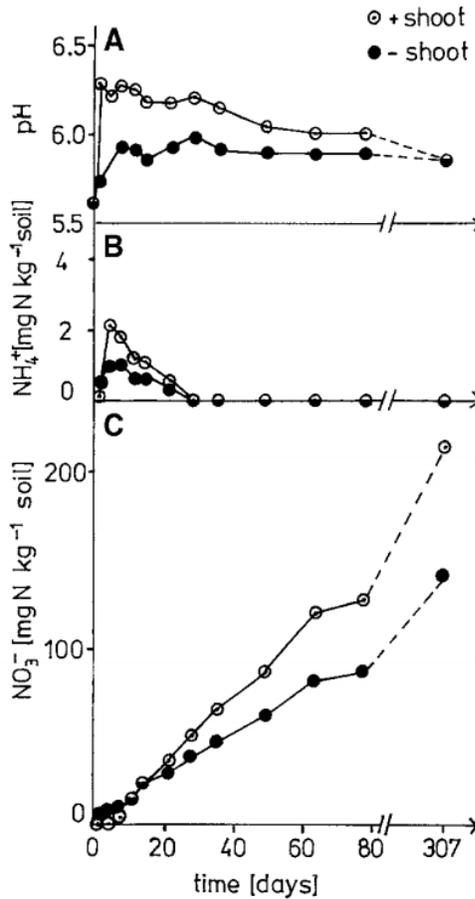


Figure 2. Changes in soil pH (A), NH₄ (B), NO₃ (C) concentrations after incorporating field bean shoots into soil (Yan et al., 1996).

4. The Relations Among Soil Reaction and Nodulation and Nitrogen Fixation

Legumes are among the most important cultivated plants worldwide. They can form new root organs known as nodules, and they maintain a symbiotic life with soil-dwelling bacteria called rhizobia in these organs. As a result of this symbiotic life, nitrogen fixation occurs. Nodule formation can be inhibited by soil pH as well as effectively

regulated by the plant. Most known legume crops can be grown in low soil pH levels. However, low pH conditions inhibit nodule development (Brett et al., 2013). Andrew (1978) stated that soil reaction (pH) is an important factor affecting on initiation of nodules and has a lesser effect on efficiency of symbiosis. He reported that Ca increase nodul formation at the soil pH between 5.0 and 6.0. However, the effect of the H⁺ ion concentration in soil is dominant over that of Ca supply with respect to nodulation at low and high soil pH levels. At lower soil pH levels, while Al excess is detrimental to nodule initiation, efficient symbiosis, plant and especially roots growth. Manganese excess does not influence nodule formation or efficiency of symbiosis but causes a significant decrease in plant growth (Andrew, 1978). Phosphorus deficiency at low and high soil pH levels reduces nodule initiation, efficiency of symbiosis and plant growth (Andrew, 1978).

It is known that there is a negative effect of low pH on the viability of rhizobia. Maximum N fixation occurs under conditions that favor rapid development of effective nodules and plant growth. While the plant is a carbon source for the rhizobia, the rhizobia is a nitrogen source for the plant. Effective nodulation is essential for optimum development and production potential of legumes planted in soils with low nitrogen content. However, the soil conditions to which the rhizobium and host plant are exposed affect the complex nodule formation processes (Burns and Norton, 2018a).

The complexity and interdependence of the processes involved in the development of effective nodules is given in Figure 3. The linkages between plant growth and rhizobia are important for root infection, nodule formation, and nitrogen fixation. Each component is affected by soil properties such as; temperature, humidity and soil pH.

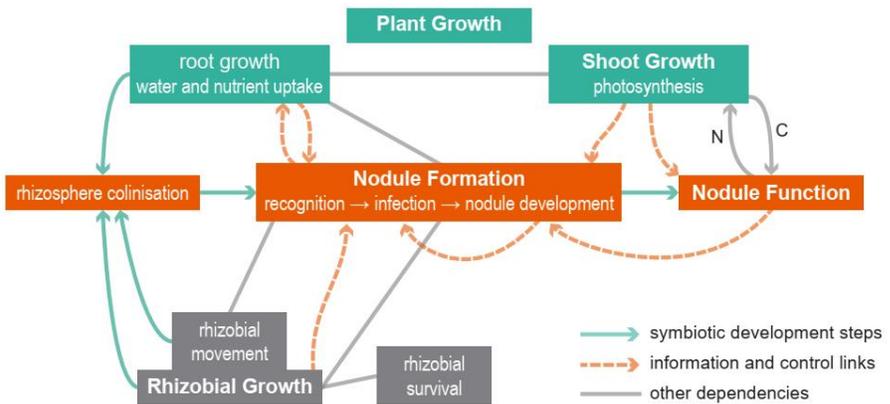


Figure 3. Lower soil pH can decrease nodulation by the detrimental impact on rhizobia, root growth and formation of functional nodules (Burns and Norton, 2018b).

The nodule development in legumes have some steps, which are sensitive to low pH, that include chemical signaling between host plant and rhizobia, accumulation of large numbers of rhizobia at the tip of host root, production of chemicals by the rhizobia, growth and infection of root hairs (Richardson et al., 1988; Abdel-Lateif et al., 2012).

Soil acidity can decrease nodulation by the negative impacts on rhizobial survival, root development and each of the steps in the interconnected sequence of events which result the formation of

effective nodules. The tolerance of legume species and associated rhizobia to soil reaction and functional nodulation are given in Figure 4.

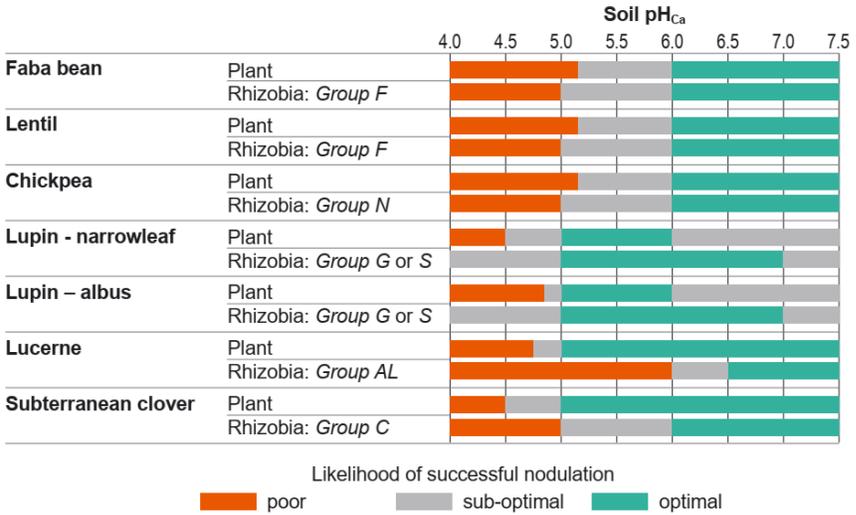


Figure 4. Tolerance of some legume species and associated rhizobia to soil pH and successful nodulation (poor, sub-optimal or optimal) (Burns and Norton, 2018b).

Soil acidity in legumes determines the mineral nutrition of plants, the intensity of nitrogen fixation, metabolic activity, product quantity and quality. Most legume plants, with the exception of lupine, grow and develop better in neutral soils. Lupine grows in soils with low pH. Some researchers (Nebolsin and Nebolsina, 1997; Valkov, 1986, Appunu and Dhar, 2006; Moiseenko and Zajtseva, 2009) have reported optimal pH values for legume plants for pea between 6.0 and 7.5, melilot between 7.0 and 8.7, alfalfa between 6.0 and 6.5, lupine between 4.0 and 5.0, alfalfa between 7.0 and 8.3, soybean between 5.5

and 6.5, raisins between 7.0 and 8.0. Neutral or weakly acid soil reactions are ideal for atmospheric N fixation. Nodules are formed at pH values varied between 4.5 and 6.0, and optimum reactions occur in a near-neutral soil pH conditions. *Azospirillum* grows better at pH ranges between 6.5 and 7.0, *Klebsiella* between 6.8 and 7.0, and *Enterobacter* around pH 7.0 (Ferguson and Gresshoff, 2015; Gao et al., 2017; Belyshkina, 2018).

As the increased acidity and alkalinity of the soil adversely affects the growth of legumes and a number of mineral nutrition processes, the structure of the stem cell cytoplasm is disrupted at pH values below 3. Under higher pH or alkaline conditions (pH 8.7), the plants are poor in NO_3^- , P, Fe, Mn, Cu, Zn contents and contain easily soluble salts (Voloshin, 2018; Nebolsin and Nebolsina, 1997). In lower pH or acidic soils, the uptake of P, Ca, K, Fe, Na and B by the stem cells is inhibited due to excess Al reducing the permeability of the cytoplasmic membrane. (Black, 1973; Stefan et al., 2018; Klimashevsky, 1991).

Zavalin et al. (2019) reported that in lower pH levels, legume productivity reduces unequally (Table 3). It indicates that melilot and alfalfa productivity are better in neutral and alkaline soils and decrease in acidic conditions, but the productivity of soybeans are smallest due to their acidophilic property.

Table 3. Changes in yield of different legumes under different soil pH values (%) (Zavalin et al., 2019)

Crop	pH value				
	4.7	5.0	5.7	6.6	7.5
Alfalfa	2	9	42	100	100
Melilot	0	2	49	89	100
Meadow clover	12	21	53	98	100
Swedish clover	16	27	72	100	95
Soybean	65	79	80	100	93

Legumes are sensitive to both hydrogen and aluminum ions. Some researchers (Jaiswal et al., 2018; Klimashevsky, 1991) reported that aluminum ions, that constitute the exchangeable acidity of podzolic soils and chernozem soils, affect plant metabolism and yield (Table 4).

Table 4. Effects of aluminum ions on various legumes (Zavalin et al., 2019).

Sensitive variety	Resistant variety
The sensitive soybean variety synthesises less organic matter per unit of nutrients (^{15}N , ^{32}P , ^{40}K).	The resistant (tolerant) soybean variety synthesises more organic matter per unit of nutrients.
Aluminium inhibits ^{32}P uptake by pea roots, and aluminium interacts with phosphates not only on the surface of root cells, but also with internal phosphorus-containing protoplasm proteins.	Resistant legume varieties isolate aluminium from roots' sensitive metabolic sites.
In the sensitive pea variety, 3 times more phosphates are fixed in the root hair zone.	In the resistant pea genotype, 66% of aluminium is localised in the root epidermis; the sensitive genotype accumulates 82% of aluminium there.

Leguminous plants are adversely affected by the high concentration of H^+ and Al^{+3} ions in the soil solution. Therefore, membrane superstructure and permeability change. Due to a change in the balance of H^+ ions in the root, membrane potential depolarization or hyperpolarization occurs, proton pump activity and K^+ and Ca^{+2} activities change which determine the formation of membrane potential, energy storage and absorption of mineral ions by the roots (Chirkova, 1988; Ivashikina and Sokolov, 2001, Stefan et al., 2018).

Petukhov (1995) reported that decrease in soil acidity to pH 6.2 increased the weight of nodules in vetch by 31% and nodules in pea by 67%; the increase of fixed atmospheric N amount were 2.8–4.0 times, and seed yield increased by 50–59%. (Table 5).

Table 5. Indices of symbiotic and photosynthetic activity in vetch and pea related to soil pH (Petukhov, 1995).

Indicator	Soil pH					
	4.6	5.5	6.2	4.6	5.5	6.2
	Vetch			Pea		
Weight of active nodules, g container ⁻¹	1.2	1.6	2.1	1.1	1.8	3.0
Lb content, mg g ⁻¹ of raw nodules	5.4	7.9	8.2	3.9	7.8	8.4
Nitrogen content in plants, g container ⁻¹	1.03	1.16	1.55	0.92	1.25	1.82
Increase in fixed nitrogen content, mg container ⁻¹	-	130	520	-	330	900
Leaf area, sq. dm container ⁻¹	18.0	20	25	13	18	23
Active symbiotic potential, g container ⁻¹	34.0	39.5	51.8	32.2	38.8	55.1
Seed yield, g container ⁻¹	7.5	9.0	14.3	10.0	13.3	19.9

Zavalin et al. (2019) determined that when the soil acidity increase, Al toxicity increases, Mg absorption decreases, sugar transport to nodules is repressed, and N fixation stops. The increases in soil pH increased the total symbiotic potential and active symbiotic potential, nodule Lb content, active nodule mass, and the amount of fixed N. After the formation of nodules, the amount of N fixation are done depend on the plant growth rate and N demand. If any factor limits plant growth, it will also limit the N fixation. In a long term, the combination of an effective liming program which increases soil pH level with neutralizing soil acidity and using acid-tolerant rhizobium can provide a sustainable solution to improve nodulation and N fixation and also increase the legume growth under acidic soil conditions (Burns and Norton, 2018b).

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CHAPTER 9

EFFECTS, TOLERANCE MECHANISMS AND MANAGEMENT OF NUTRIENT DEFICIENCY IN GRAIN LEGUMES

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INTRODUCTION

The balance of the world, which has the potential to meet the needs of human beings, is deteriorating day by day. In time, people have begun to dominate nature in order to increase their welfare level, have been in a constant struggle with the environment where they live by using technology, and have created an artificial environment by changing the environmental conditions as they wish (Türk, 1998; Kıvanç and Yücel, 1998; Özey, 2001; Şama, 2003; Baysak, 2008).

The interest in the land increased even more with the transition of humanity to a settled life with agriculture. The first artificial fertilizers used in history were sodium nitrate and bones. At the beginning of the 19th century, when agricultural chemistry related to the nutrition of plants began to develop, the principles of fertilizer and fertilization began to be determined. At the end of the second world war, very rapid development was observed in the nitrogen fertilizer industry, which was far behind the phosphorus and potassium fertilizer industry at the beginning of the twentieth century. The widespread use of chemical and animal fertilizers containing nitrogen and phosphorus without care has become one of the most common human-made environmental pollution causes on soil and water today (Alpates, 2014; Güneysan, 2014).

As a result of the change in the natural structures of the atmosphere, hydrosphere and lithosphere that make up our world, these places have begun to cease to be a living environment, and with the deterioration of the relations between the living and non-living beings that make up the

ecosystem, ecological problems known as economic, social and environmental problems have emerged (Uruç, 2006).

Only 1/10 of the land in the world can be produced. Approximately 36% of Turkey's land assets are cultivated. When the change in Turkey's agricultural lands by years is analyzed, it is seen that the total agricultural areas decreased by 6.93% in 2008 compared to 1990 (Bayramoğlu, 2010).

One of the important causes of ecological problems, especially environmental problems, experienced by people is the rapid increase in population. With the industrial revolution, the population of the world entered a rapid increase process in the 18th century and the population growth rate was 0.8% until 1950, and it increased to 1.9% between 1950-1985. It is predicted that the world population will increase to 8.2 billion in 2025 and 10.2 billion in 2050 (Yıldız et al., 2000; Uruç, 2006).

It is necessary to switch to sustainable agriculture to prevent the damage of agriculture to the environment, to use agricultural inputs consciously and less, to spread organic agriculture and to meet the needs of future generations. Urban areas threaten agricultural areas, destroy forest areas, pollute potable water resources; The increase in such threats with the effect of industrialization ensures that people are faced with the risk of destroying their food security and nutrition opportunities. Such risks pose multifaceted problems in the physical and social environment in human societies. As revealing factors, they put the vital resources of today's world societies into danger (Geren and Altan, 2005; Deveci, 2014).

Purpose in agricultural production; the most effective factor for obtaining the highest possible yield and quality product; to make balanced and regular fertilization. This is only possible with soil analysis. With soil analysis, the deficiencies of nutrients that will enable the growth and development of the plant in the soil to be produced are determined. According to the results of the analysis, it is determined how much, when and how to give which fertilizer (Taban, 2014).

Food supply and consumption habits are changing rapidly. The passion of changing the world or protecting it as it once was, has been replaced by the concern of ensuring security (Furedi, 2014). Food safety means sufficient food intake and consumption to maintain a healthy, productive and productive life today and in the future (Hatlemitoğlu, 2006). Safe food is defined as food that is suitable for consumption and has not lost its nutritional value in terms of its physical, chemical and microbiological properties. It is of social, economic and environmental importance both for the country and for the producers and consumers of other countries on a global scale (Ertunc, 2010).

The food safety issue is a national and global concept. It is of social, economic and environmental importance for producers and consumers of other countries, both in the country and globally. Consumers are no longer like the old consumers; they are more conscious. Besides wanting to know what they are consuming, they act more selectively; want to consume good and healthy food (Ertunc, 2010).

Although more than two decades have passed since the First World Food Conference, the importance of safety in the world's food sources,

more than quantity, has never changed. Food safety starts at pregnancy; It continues with adults, children and babies fed under suitable conditions. Advocates of breastfeeding see breastfeeding as part of their national food safety strategy. Genetically, human proteins were transferred to the first transgenic dairy calf, Herman, and studies were conducted to create a biological environment with human genes to produce milk with human protein value (Hatlemiçođlu, 2006).

Nutrition is an indispensable requirement not only for humanity but also for all living things. Vegetable and animal foods, especially high in protein, have great importance in nutrition. Legumes have a distinct advantage over other nutrients in terms of elements such as calcium, iron, phosphorus and vitamins such as B1, B2 and niacin. Legumes are a major source of protein, an important dietary product, and a source of nutrients that protect soil and water resources. 22% of vegetable proteins and 7% of carbohydrates in human nutrition are provided from legumes. additionally, legumes also have various health care properties. So, legumes should be used in crop rotation programs and in diets two times per week to achieve sustainability of food security, soil management and environment. In this review; legumes and plant nutrients, which have an important place in the nutrition of living things and sustainable agriculture, are mentioned.

FERTILIZATION AND MANAGEMENT OF NUTRIENT DEFICIENCY IN GRAIN LEGUMES

The continuous increase in the world's population and the diversification of human needs in parallel with this increase create

constant pressure on land use. As a result of these pressures, important problems gradually arise in lands that are misused (Donma, 2008).

Many processes that need to be done to increase productivity in agricultural production have now become feasible. Despite many remedial actions such as the regulation of fertilization programs based on soil analysis, the widespread use of high-yielding and disease-resistant breeding varieties, and improvements in irrigation systems, there are still studies to be done. One of them is to determine the organisms that exist in the nature of the soil, to determine the ones suitable for use in agricultural production and to make them available in the market by making the necessary laboratory and field studies. The organisms known and most commonly used in this field are *Rhizobium* bacteria, followed by Mycorrhizae. In addition to these, the use of microorganisms called Plant Growth Promoting Rhizobacteria is becoming widespread. These include a group of organisms such as nitrogen fixers, phosphate solvents, potassium solvents, bacteria, fungi and actinomycetes (Sönmez, 2012).

With the efforts of people who are sensitive to the environment and human health, the necessity of staying away from these kinds of drugs and chemical fertilizers in order to contain maximum vitamins in a product is tried to be explained by various studies. Although it is difficult to create a common awareness on this issue, organic farming practices have started to be preferred by reducing the use of chemical inputs in the regions where the studies have been successful (Kırmacı, 2003).

Chemical use disrupts the soil structure and causes erosion. Input-intensive production method in conventional agriculture has an increasing effect on the threat elements on the soil. The use of pesticides, as well as the use of chemical fertilizers, makes it difficult to preserve the soil (Hathaway-Jenkins et al., 2010; Sahin and Ceritoglu, 2020).

Nitrate, which is mixed with water by fertilization or can accumulate in the plant, is the main polluting element. Drinking water should not contain nitrate nitrogen at a level higher than 20 mg/kg. The increased use of nitrogen fertilizers can cause ammonia and nitrous oxide emissions, which adversely affect the air. In addition, nitrous oxide gas passing into the atmosphere causes the ozone layer to decompose. As a result of excessive nitrogen fertilization, a significant accumulation of nitrate and nitrite is observed in plant tissues, and this can cause significant health problems in humans and animals fed with these plants (Karaca and Turgay, 2012).

The regions where fertilizer consumption is most intense in our country are the Mediterranean, Marmara and Aegean Regions. Excessive use of phosphorus fertilizers causes Cd accumulation in agricultural soil and plants. Cadmium is the element with the highest solubility in the water among other heavy metals (İkizoğlu, 2008).

Root and root hairs, morphology (length, root density and root surface area), H^+ , OH^- and HCO_3^- extraction of roots, organic acids of roots such as citric acid, malic acid, tartaric acid and oxalic acid and phenolic acid are among the plant factors in microelement nutrition of plants.

They reported that extraction, sugar and protein-forming amino acid content of roots (phytosiderophore), secretion of phosphatase enzyme of roots, plant demands, plant species and varieties, carbon dioxide production of roots, mycorrhizal lifestyles of plants are important (Fageria et al., 2002).

Bioplastics, known as plastics, obtained from polymers of biological origin or renewable carbon sources; They are biological materials produced by various organisms such as animals, plants, fungi, algae, or bacteria (Luengo et al., 2003; Rajendran et al., 2012; Özdemir and Erkmen, 2013). Biomaterials; They are natural products synthesized by various organisms by biotechnological methods and easily assimilated by many living things compared to conventional synthetic products, and they do not have a toxic effect on organisms because they are biocompatible (Vroman and Tighzert, 2009; Özdemir and Erkmen, 2013). Because of these properties, bioplastics are also known as a special form of biomaterials produced by microorganisms in a wide range of different foods and environments (Luengo et al., 2003; Özdemir and Erkmen, 2013).

Today, bacterial species that promote plant growth have been identified; *Azospirillum*, *Azotobacter*, *Agrobacterium*, *Acetobacter*, *Aereobacter*, *Alcaligenes*, *Artrobacter*, *Acinetobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Clostridium*, *Chromatium*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Klebsiella*, *Pseudomonas*, *Rhizobium* and *Serratia* (Bashan and de-Bashan, 2005). Endophytic PGPBs have been detected in leaf tissues. The mechanisms by which PGPB promotes

plant growth are not fully understood. However, some important data have been obtained on this subject. For example; production of plant hormones such as auxin (Egamberdiyeva, 2005), cytokinin (Garcia de Salamone et al., 2001), gibberellin (Gutierrez- Manero et al., 2001) and inhibition of ethylene production (by ACC deaminase activity) (Glick et al., 1995), asymbiotic N₂ fixation (Sahin et al., 2004), solubilization of inorganic phosphate and mineralization of organic phosphate and other nutrients are the most important ones (Jeon et al., 2003). PGPB also indirectly promotes plant growth by preventing the harmful effects of phytopathogenic microorganisms (bacteria, fungi and viruses) (Van Loon and Glick, 2004). By reducing the amount of ethylene synthesized as a result of stress conditions with the ACC deaminase enzyme it produces, PGPB provides resistance to the destructive effects of excessive ethylene production in the plant (Burd et al., 1998; Grichko et al., 2000; Wang et al., 2000; Grichko and Glick 2001; Mayak et al., 2004a,b; Kausari and Shahzad, 2006; Nadeem et al., 2007; Arshad et al., 2008).

The term PGPR, which is an abbreviation of the words “Plant Growth Promoting Rhizobacteria”, was first used in 1978 (Kloepper and Schroth, 1978). Root bacteria that stimulate plant growth are generally found in genera such as *Bacillus*, *Lactobacillus*, *Paenibacillus*, *Arthobacter*, *Pseudomonas*, *Burkholderia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Xanthomonas*, *Serratia*, *Rhizobium*, *Bradyrhizobium*, *Azospirillum*, *Azotobacter*. Numerous studies on PGPRs have been carried out in many countries of the world since 1978. For many

products, an average yield increase of 10-22.5% was obtained (Uslu, 2006). Many of the PGPR group bacteria can also work as a very good biological warfare element. These bacteria can provide significant success in the fight against plant diseases, especially soil-borne diseases. In this regard, there are many examples in the world within the concept of biopesticides.

When PGPRs are considered both in terms of their productivity-enhancing properties, as biofertilizers, and as biopesticides in biological warfare, as mentioned above, PGPRs are considered as indispensable elements at the center of agricultural techniques such as Organic Agriculture and Integrated Product Management, which are very popular today. It was a very important development in the 1960s to use chemicals such as pesticides and fertilizers to meet the nutritional needs of the growing world population, to increase productivity in agricultural production and to reduce losses caused by disease factors and pests. This process, which provides many benefits to agricultural production, is called the "Green Revolution". However, it did not take long to see the benefits of the use of chemicals in agriculture and the negative effects on human and environmental health. Even today, there is great public opinion in the world against the use of chemicals. This has been increased research on reducing the use of chemicals in agriculture and developing alternative methods, and the emergence of new concepts such as organic agriculture, sustainable agriculture, or integrated product management in terms of agricultural production techniques (Tilak and Annapurna, 1993; Tilak et al., 2005). At this point, the use

of biological elements gains great importance in increasing plant productivity and preventing yield losses in plants caused by diseases or pests. The use of biotechnological products in agriculture is attracting increasing attention in the world (Whipps, 2001; Vessey, 2003; Kloepper et al., 2004; Ping and Boland, 2004; Barea et al., 2005; Tilak et al., 2005).

It seems that today and, in the future, the use of these products in agriculture will become increasingly widespread, and a period based on the dominance of a new trend, perhaps called "Bio Revolution", will begin. Today, in the context of increasing productivity, some applications are made in agricultural production, mostly with beneficial microorganisms, which are of biological origin. The most common example in this regard is the use of symbiotic rhizobium, which fixes the free nitrogen of the air in legume production. The first application of commercial *Rhizobium* inoculants for use in legumes coincides with the 1890s (Vessey, 2003; Uslu, 2006).

As a result of studies carried out in different parts of the world, yield increases have been recorded in many products. According to studies conducted in the United States, 5-20% in Barley; 57% in Canola; 14-25% in peanuts; 12-24% in paddy; 12-15% yield increase was achieved in celery (Kloepper et al., 1991). While the first studies on PGPRs were limited to tuberous crops such as radish, potato and sugar beet, later they included barley, rapeseed, cotton, peanuts, lentils, peas, beans, chickpeas, rice, soybeans, vegetables and tree species such as gladiolus, apple and citrus fruits. It has also expanded to include (Kloepper, 1992).

Increasing amounts of PGPR products are being marketed in the world, and it is stated that approximately 65% of the nitrogen support worldwide is met with biofertilizer applications (Matiru and Dakora, 2004; Varma et al., 2004). Many symbiotic, diazotrophic bacterias have been tested and identified as biofertilizers (Barea et al., 2005). As mentioned before, PGPRs are found and used as commercial bioinoculant products in China, many European countries such as the USA and India (Hetong, 2004; Matiru and Dakora, 2004; Varma et al., 2004; Barea et al., 2005).

Among the direct stimulation mechanisms of PGPRs, the production of plant hormones such as auxins, gibberellins and cytokinins (plant growth regulators) by bacteria, regulation of their concentration in the rhizosphere (such as regulation of ethylene synthesis by ACC-deaminase), or stimulation of their production in the plant (Cattelan et al., 1999; Almonacid et al., 2000; Gray and Smith, 2005; Ping and Boland, 2004; Barea et al., 2005; Tilak et al., 2005); Increasing plant nutrients in the soil (via nitrogen fixation) or converting them into forms that the plant can benefit from (iron via siderophores, phosphorus with organic acids or phosphatases) (Cattelan et al., 1999; Whipps, 2001; Tilak et al., 2005) are counted. PGPRs are indirectly affected by biocontrol mechanism to plant growth by preventing diseases caused by fungal, bacterial and viral plant pathogens and minor pathogens that negatively affect plant growth, in other words, harmful soil microorganisms (DRMO) and even by reducing the damage of abiotic factors or insect and nematodes. (Van Loon, 1997; Whipps, 2001; Singh

et al., 2003; Van Loon and Glick, 2004; Varma et al., 2004; Li et al., 2005; Tilak et al., 2005; Ji et al., 2006; Umesha, 2006). Kloepper (1992) likens the plant growth stimulating activity and biocontrol effect of PGPRs to two sides of a coin. In addition, it is stated that some PGPRs prevent their negative effects on plants by accumulating heavy metals in soils containing heavy metals such as Zn, Cd, Pb and Ni, which cause toxicity to plants (Varma et al., 2004; Barea et al., 2005).

The higher the number of living things in the soil, the more fertile the soil. Microorganisms need nutrition and energy in order to survive, the main source of which is organic matter. Organic substances regulate the structure of the soil. They increase the air and water permeability, workability and looseness of the soil. They prevent infertile clay soils from breaking down into fertile soil and from compaction at the same time, keeping them by more air and fluff. They prevent erosion by increasing the ability of the colloids in the soil to hold each other (Saltalı, 2014).

It has been reported in many studies that many bacteria living symbiotically with plants provide significant gains to the plant by living in the root rhizosphere, rhizome and leaves of the plants. These are called "Plant Growth Promoting Bacteria" (PGPB) "Plant Growth Promoting Bacteria". These bacteria have been used as bio-fertilizer in agricultural areas in recent years. Synthetic fertilizers; It is clearly known that it causes important problems in soil, plant, environment and even human health. On the other hand, it is claimed that bio-fertilizers with PGPB will reduce these problems and make a significant

contribution to the protection of natural environments. PGPBs increase plant growth with effects such as nitrogen fixation, plant hormone production, bacterial siderophore production, ensuring the uptake of iron and similar elements, phosphate dissolving, synthesizing stress-tolerant compounds, and suppressing plant pathogens. In particular, studies with important cultivated plants have shown increasing effects on plant growth and yield (Selvakumar, 2007; Turan et al., 2013).

The fact that chemical fertilizers used in crop production cause environmental problems have increased the importance of alternative applications that provide product increase. The unknown world of microorganisms in soil ecology has been the most interesting part of these applications. By using new technological methods, the events occurring in the microorganism world and the microorganisms involved in them can be identified more rapidly and they are tried to be used in agricultural production. The existence of microorganisms that can create an alternative to the use of intensive chemical fertilizers also requires investigation of their use in agriculture (Sönmez, 2012).

The intensive use of chemical fertilizers is a factor of environmental pollution. Instead of using excessive phosphorus fertilizers to meet the phosphorus needs of plants, it is also important to ensure that they benefit from the existing phosphorus in the soil through microorganisms, as it contributes to the prevention of environmental pollution.

Phosphate deposits in our country are 300-400 million tons and are generally located in the Southeastern Anatolia region, 70 million tons

of this reserve is workable. Due to the low phosphorus grade, the desired production cannot be achieved and accordingly, imports are used to meet the country's demand (Demir and Yalçın, 2004). Vassilev et al. (2006) reported that as a result of in vitro studies, phosphate-dissolving bacteria, secreted by the siderophore, phytohormones and lytic enzymes, improve plant growth and phosphorus uptake, as well as suppress disease-causing organisms.

Many studies have been carried out for 200 years to analyze the chemical structure and origin of humic substances, but no clear conclusion has been reached. There is no second substance in the history of chemistry where the structure of a substance could not be fully resolved in such a long time. In this historical process, concepts for humic substances have been put forward, analysis methods have been developed, and serious discussions have been made about their structure and origin (Dizman et al., 2012). The chemical elements present in the soil are not always in sufficient quantity, even if they are in sufficient quantity, they are not in the form that can be taken up by the plants. The most important problem in providing optimum growth for plants is the lack of necessary elements in the soil that the plant can use when it needs it. The main content of organic matter in the soil is humic matter, and the most active biochemical substance of the humic matter is humic acid. Unfortunately, the developments in modern agricultural practices have increased the use of chemical fertilizers and caused the rapid depletion of the humic substance that facilitates the uptake of these fertilizers. As a result, the need to use fertilizers every

year for efficiency has arisen. However, in recent years, the importance of humic substance applications to increase the amount of soil organic matter in modern agriculture has begun to be understood (Anonymous, 2010). Humification can be considered a slow and stable reaction (Dizman et al., 2012).

Since it contains plant nutrients, its toxic element content is low and its humic acid content is high, most of the researches carried out in our country has focused on the potential for use of leonardite as a soil conditioner. Studies continue on subjects such as the effect on plant yield, organic matter content and evaluation of humic substance content (Engin et al., 2012). It is stated in the studies that there are humic and fulvic acids in all organic substances found in nature. However, according to the studies done so far, the organic matter containing the highest humic and fulvic acids with a ratio of 40-90% is incompletely lignitized brown coal (young lignite), also known as leonardite (Gezgin et al., 2012).

For big cities, organic fertilizers can be obtained by separating organic garbage from other garbage and recycling it. If tobacco dust released in tobacco factories is composted, it becomes organic fertilizer (Karaman et al., 2012a). In addition, garbage, domestic and industrial wastes, park, garden and marketplace wastes, street wastes, agricultural wastes, treatment sludge are used, and all organic wastes should be evaluated at different rates. Advantages of compost making; It provides various nutrients and N to the soil, improves air and water inlet and outlet, and accelerates plant root development. It improves the physical, chemical

and biological properties of the soil. Since it is easy to store, it can be applied at any time of the year (Cooperband, 2002).

One of the renewable energy sources is biogas. Various animal manures can be used alone or mixed with other organic wastes as a raw material for biogas production, and also all organic waste such as tree, corn, wheat plants, algae, fruit and vegetable waste from houses, animal excrement, manure and industrial wastes can be counted. The liquid-solid part remaining after biogas production is used as fertilizer. Biogas is a renewable energy source that has no disadvantages, and the biogas plant does not cause any harm and discomfort to the environment and people (Coşkun et al., 2011).

As a result of the production surplus experienced recently and the destruction of natural resources, which are increasingly damaged, the formation of excess production in many products causes the products to be sold at less than their value. Damages to natural resources are increasing in the whole agriculture sector. Growing organic agricultural products is seen as a very effective tool for the efficient use and protection of natural resources and is rapidly increasing its importance all over the world. Thanks to organic agricultural production techniques, the danger of erosion is prevented by minimizing soil loss. In addition, the protection of human health can be ensured by the consumption of organic products (Gok, 2008).

The concept of naturalness is within the context of organic agriculture. The concept of naturalness is frequently mentioned, especially in comparisons of organic agriculture and traditional agriculture. The

main element of natural production is the avoidance of the use of chemical fertilizers. The fact that external factors such as pesticides and hormones are not included in the production of organic agriculture causes the products to be natural. With the concept of naturalness, the effectiveness of family business is ensured and other living creatures and the environment are protected (Doğan, 2017).

Many studies show that CO₂ emissions are 15-20% less in organic farming than in conventional farming. The most important reason for this is that inorganic N is not used in organic agriculture. Soil has twice as much carbon as the atmosphere. The use of inorganic fertilizers in agriculture and the high production level may cause more organic matter loss from the soil. It is aimed to protect and increase the organic matter of the soil in different ways. It has been estimated that if the soil is developed as a CO₂ bank, the gases that may emerge from fossil fuels in 15 years will be absorbed by the soil (Rehber, 2011).

The share of the low productivity potential of our soils is very high in the yield and quality of crop production in our country. This situation is one of the most important causes of income loss and the economic inadequacy of farmers. One of the ways to solve this problem is to increase the organic matter content of the soils and increase their productivity potential. In this context, vegetable and animal residues such as barnyard manure, vegetable residues (stubble), green manure, composts and leonardite should be used intensively in our agricultural areas. However, since it is not possible to add sufficient amounts of organic fertilizers to all our soils, the fertility potential of our soils can

be increased by applying humic and fulvic acids, which are the active fractions of organic matter and humic matter, in much smaller amounts than organic fertilizers. Because the main reason for the positive effects of organic fertilizers on soil properties is many organic compounds released by the decomposition and decomposition of microorganisms in the soil and the humic substance whose structure is formed by humic and fulvic acids (Alak, 2014).

Physical effects of humic acids on soil; It increases the water holding capacity of the soil, the aeration of the soil and the workability of the soil, provides resistance to drought and reduces soil erosion. Chemical effects; Keeping water-soluble chemical fertilizers in the root zone, releasing them when plants need them, transferring plant nutrients to the form that plants can take, having high ion exchange capacity, contributing to the deterioration of rocks and minerals, increasing the buffering balance of the soil, chelating ions in alkaline conditions, necessary for plant growth It provides both organic and inorganic matter. Biological effects; By affecting cell division, it accelerates seed germination and plant growth, increases root growth rate, dry matter yield, the permeability of plant membranes and facilitates the intake of plant nutrients from the leaf. It provides root development, the survival and development of soil microorganisms, and helps photosynthesis. As a result, these positive effects of humic and fulvic acids on the physical, chemical and biological properties of soils, significantly increase plant yield and quality by increasing the germination of plant seeds, the development of roots and above-ground parts and flowering, better

utilization of soil water and air and balanced nutrition. In addition, humic and fulvic acids contribute to the economy by increasing the effectiveness of chemical fertilizers and preventing the use of excessive fertilizers, as well as helping to protect the environment (Anonymous, 2013).

Chemicals used in agricultural activities disrupt the balance of nature and cause negative effects on public health. The move away from the use of chemicals in agriculture and the increasing interest in organic agriculture increase the importance of leonardite raw material and humic and fulvic acid obtained from this raw material in agricultural activities. Leonardite is an organic substance formed by the exchange of lignite in the air atmosphere. It did not complete the charring process, was exposed to high oxidation conditions and eventually reached a high humic acid content. The most important difference that distinguishes Leonardite from lignite is that it has a high percentage of oxygen and does not have the opportunity to use it as a fuel (Engin et al., 2012).

While low molecular weight humic substances (fulvate) in leonardite (organic humic matter) are closely related to chemical reactions affecting the metabolic processes of plants, high molecular weight humic substances (humate) change the physical characteristics of the soil. Numerous scientific research results have revealed that the availability of macronutrients increases and adjusts the absorption of micronutrients by plants, depending on the application of humic substances and the chemical interactions of humic substances in the soil (Karaman et al., 2013).

In plant cultivation, it is emphasized that potassium plant nutrient has an important place on plant root development and product amount, the plant's resistance to cold, plants' resistance to lodging, on the harvest time, the effectiveness of nitrogen, the resistance of plants to diseases and plant quality. It was determined that photosynthesis, ribulose diphosphate carboxylase enzyme activity and photorespiration increased as potassium content increased in plant leaves. According to Fageria (2009), as explained before, potassium causes an increase in protein content, increasing the nutritional value and quality of food and fodder plants. According to some researchers, potassium increases the resistance against diseases by thickening the outer walls of epidermal cells. Potassium deficiency occurs with growth reduction in young maize plants, the leaf edges first turn yellow, then turn into brown necrotic tissue in a striped appearance. Due to the weak root development, the plant lies against the slightest interaction (Kacar, 2012).

More than 50 kg da⁻¹ of nitrogen is provided annually in pastures where legumes are abundant (Boşgelmez et al., 2000). In addition, nitrogen gain with green manure is not only valid for that year but continues for several years. It has been determined that 2-10 kg da⁻¹ of nitrogen is provided to the plants with green manure (Vuralın, 2010).

The results obtained from the trials established with Eresen 87 broad bean varieties, it is possible to get about 4-5 tons da⁻¹ of yield and 1 ton da⁻¹ of dry matter yield in the broad bean agriculture in Ödemiş Plain, it is important for animal husbandry as protein and mineral matter, and

the bean is green It has been observed that it enriches the soil by adding more than 25 kg of organic nitrogen per decare as a result of its evaluation as a fertilizer (Geren and Altan, 2005).

Doğan et al. (2007) investigated the effect of bacterial inoculation and iron applications on nodulation and plant nitrogen uptake in peanut plants and determined that bacterial applications statistically increased the nitrogen content, nodule number and nodule weight values of the plant. They observed that iron application significantly increased the nodule nitrogen content.

The effects of rhizosphere microorganisms on the growth, yield and nutrient content of chickpeas were investigated (Zaidi et al., 2003, Zaidi and Khan, 2007), as a result of their pot study, that high plant growth, yield and nutrient uptake were determined by *Mesorhizobium ciceri* and phosphate-solubilizing bacteria *Serratia* or phosphate-dissolving fungus *Penicillium*. Reported that they achieved in their application together. Akhtar and Siddiqui (2009) investigated the effects of Rhizobium inoculation with phosphate-dissolving microorganisms on the development of chickpea and root rot disease. As a result of the study, they reported that rhizobium inoculation alone provides more yield and growth, but the most important effect against root rot disease is provided by *P. Putida* phosphate solvent organism. Pramanik et al. (2009) reported that rock phosphate soil treated with vermicompost showed higher phosphorus mineralization than other applications in their study with vermicompost applied and unapplied rock phosphate. Ndung'u-Magiroy et al. (2012) determined that there is a significant

relationship between phosphate-solubilizing bacteria and soil organic carbon, exchangeable calcium and exchangeable magnesium contents at the $P < 0.05$ level in their study in Kenya.

It has been observed that stress is reduced by microbial inoculation against abiotic stresses such as drought, freezing, temperature, salinity, and metal toxicity in plants (Hontzeas et al. 2006; Cheng et al. 2007; Nabti et al. 2010). Many studies have shown that plant growth and regulatory rhizobacteria (PGPR) make plants tolerant to stresses such as drought, salinity, and metal toxicity (Esitken et al. 2006; Turan et al. 2006, 2007).

Ece et al. (2007) investigated the effects of nitrogen and phosphorus fertilizer amounts and leonardite applications in the study named “Effects of Leonardite Applications on Pollen Bean Yield and Some Soil Properties”. As a result of the study, the effect of leonardite application on the EC, pH and lime contents of the soils was not observed and the organic matter, nitrogen and phosphorus content of the soils increased compared to the control application. The bean yield showed the highest value in $13 \text{ kg N da}^{-1} + 10 \text{ Kg P}_2\text{O}_5 \text{ da}^{-1} + 1\text{-ton leonardite application}$.

In a study (Genişel, 2010), CaCl_2 and bone powder solution applications showed that the protein amounts, photosynthetic pigment contents, antioxidant enzymes (CAT, POD and SOD) in the leaf tissue of the bean (*Phaseolus vulgaris* L.) plant exposed to salt (NaCl) stress. investigated the effects on lipid peroxidation and hydrogen peroxide amount. NaCl application caused a decrease in the protein content of

the plant leaf tissue. However, bone powder solution and CaCl_2 applications at different concentrations had a positive effect on protein content. The effect of bone powder solutions on protein content was found to be higher than the effect of CaCl_2 applications.

In the study, where 98 dry bean cultivars of Göynük were used as material (Yiğitarıslan, 2010), two different doses of kaolin solution (3% and 5%) were applied to the leaf surface of the plant at 3 different times (vegetative period, pre-bloom, pod binding period). According to the findings, the protein ratios were determined as 20.11%, 20.15% and 20.17% in the vegetative period, before flowering and pod-seeding period, respectively, in the control cultivars, while the protein ratios in the 3% Kaolin application were 20.88%, 20.77% during the vegetative period, before flowering and pod-seeding period, respectively. and 21.07%. In the application of 5% Kaolin in beans, it was determined that the protein ratios were 21.32% in the vegetative period, 21.37% in the pre-flowering period and 21.46% in the pod setting period.

In a study (Ceyhan et al., 2006), 6 types of dwarf dry beans were used. In the field experiment they carried out on soil containing 0.19 mg kg^{-1} boron, 3 different boron doses were given to the plants from the soil and leaves (control, 3 kg ha^{-1} soil and 0.3 kg ha^{-1} of leaves), and as a result of the application, they determined that bean varieties showed a wide genetic variation in terms of bora responses, the grain yield increased by 10% to 20% with the application of boron to bean varieties, and the highest increase was in soil application of 3 kg ha^{-1} boron. They found that the highest grain protein ratio was in Karacaşehir - 90 variety, and

the lowest protein ratio was in Önceler - 98 variety, depending on boron application.

Nadergoli et al. (2011) determined that two microelements (Zn sulphate and Mn sulphate) and their application methods in 2008 and 2009 growing seasons in Iran, examined the effect on yield and components of beans. They reported that the highest seed in the pod, pod in the plant, yield and components were obtained from microelement applications. They reported that this application increased these features by 37.07%, 44.74%, 45.43%, 7.04%, 75.1% and 13.69% compared to the control. They reported that the highest 1000-grain weight was obtained from the magnesium sulfate application applied during the stem, flowering and pod periods, and the highest number of immature seeds was obtained from the zinc sulfate application.

In a study (Şanlı, 2007), Gökçe and Akçin 91 varieties and Spanish chickpea population were used as material. In the experiment, 3 different sowing times (31 March, 16 April and 02 May 2006) and 6 seed treatments (control, distilled water, 100 ppm GA3, 200 ppm GA3, 300 ppm GA3, 400 ppm GA3) were applied. According to the results of the analysis of variance made with the data on the characteristics discussed, significant differences were determined in terms of varieties, sowing times and seed applications, and the crude protein ratio was found to vary between 20.1-27.3 values.

In a study (Yağmur and Engin, 2005a), in their study conducted in Van between 1997-1998, different phosphorus (0, 3, 6, 9 kg P₂O₅ da⁻¹), nitrogen (0, 2, 4, 6 kg N da⁻¹) doses and the effect of inoculation with

Rhizobium ciceri (inoculated and non-inoculated) on yield and yield components of ILC 482 chickpea (*Cicer arietinum* L.) variety were evaluated. As a result, they reported that the nitrogen doses applied in both years had a significant effect on the grain yield, biological yield, crude protein ratio and grain yield in the plant. Researcher Yağmur and Engin (2005b) stated that the grain yield averages varied between 103.49 kg da⁻¹ and 122.75 kg da⁻¹ at different dose levels of phosphorus in 1997, and between 64.57-78.96 kg da⁻¹ in 1998, and high grain yield 6 kg N da⁻¹ dose (respectively 132.87 and 87.95 kg da⁻¹) reported that they detected.

Researchers (Meyveci et al., 2002), in their studies investigating the effects of zinc and iron fertilizers on yield in chickpea; In general, reported that zinc fertilization for chickpeas provides a certain increase in yield depending on the varieties, iron fertilization is less effective in yield compared to zinc fertilizer, especially when zinc + iron fertilization is given together, there is no increase in yield compared to control.

Researchers (Khorgamy and Farnia, 2009) investigated the effect of phosphorus and zinc fertilization in chickpea under dry conditions in Iran. Researchers reported that phosphorus and zinc applications had a significant effect on plant height, number of main branches, 100-grain weight, seed yield, biological yield, number of sub-branches, number of nodes in the main branch, zinc concentration and protein concentration.

Valenciano et al. (2010) found that in chickpeas, zinc (0, 1, 2, 4, and 8 mg Zn pot⁻¹), boron (0 and 2 mg B pot⁻¹), and molybdenum (0 and 2 mg Mo pot⁻¹) in their studies in which they investigated the effect on yield and nutritional elements; reported that chickpea reacted to the microelement application, the total dry matter production was higher, the highest harvest index (60.3%) was obtained from Zn₄ × B₂ × Mo₂ application and the lowest harvest index (47.65%) was obtained from Zn₀ × B₀ × Mo₀ application. They reported that Zn, B and Mo applications improved seed yield as they increased the number of pods per plant, and the highest yield (4.00 g plant⁻¹) was obtained from Zn₄ × B₂ × Mo₂ application.

Togay et al. (2001), used three lentil cultivars (Sazak-91, Yerli Kırmızı and Kışlık Kırmızı-51) at different doses (0, 1, 1.5 and 2 kg da⁻¹) of zinc yield and investigated the effect on yield items under Van ecology. The average maximum grain yield of two years was 71.36 kg da⁻¹ from Sazak-91 cultivar with 2 kg da⁻¹ zinc dose application. As the zinc doses increased, an increase was observed in the grain yield per unit area, and the optimum zinc dose for all cultivars was 2 kg da⁻¹.

The researcher (Gulser, 2004) determined the nutritional content of three lentil cultivars (Sazak-91, Yerli Kırmızı and Kışlık Kırmızı-51) of 5 different doses of zinc (0, 0.5, 1.0, 1.5 and 2.0 kg Zn da⁻¹) in 1998 and 2000. in their research in which they determined the effect; reported that the highest grain yield was obtained from the highest zinc application in cultivars in areas with zinc deficiency and grain yield increased as the zinc doses increased. Additionally, researchers

(Yağmur and Kaydan, 2005) reported that doses of foliar fertilizer containing macro and microelements increased the grain yield per unit area in the Sazak-91 variety.

Researchers (Zeidan et al., 2006), in 2003 and 2005 (in Al-Nagah village, in El-Tahrir province), investigated the effect of Fe, Mn and Zn microelements on poor sandy soils due to the decrease in yield in lentils due to microelement deficiency. They reported that the formation of chlorosis was prevented by Fe, Mn and Zn applications, and the plant height, the number of pods and branches in the plant, and the thousand-seed weight increased with N, P and K applications.

Togay and Anlarsal (2008a) reported that different doses of zinc (0.0, 1.5, 3.0 and 4.5 kg da⁻¹ ZnSO₄.7H₂O) and phosphorus (0, 2, 4, and 6 kg P₂O₅ da⁻¹) were given to lentils in Van conditions in 2000 and 2002. In their research, they carried out in order to investigate the effect of yield and yield elements on the cultivars and their uptake by the plant. In the first year, the highest yield was 95.11 kg da⁻¹ of Sazak-91 with 1.5 kg da⁻¹ of zinc and 4 kg da⁻¹ of phosphorus, and in the second year with 198.70 kg da⁻¹ of Sazak -91 with 4.5 kg da⁻¹ of zinc and 4 kg da⁻¹ of phosphorus. reported that their application.

Togay and Anlarsal (2008b) investigated the effects of different doses of zinc and phosphorus on yield and yield components in winter red lentil cultivars in Van conditions. It has been reported that different applications of zinc and phosphorus doses have the highest and lowest values, although they vary over the years on all the characteristics examined, this may be due to zinc intake is prevented at a high

phosphorus ratio and the two nutrient elements create a competitive environment.

Ergün and Öncel (2010) investigated the toxic effects of cadmium (Cd) and zinc (Zn) with a concentration of 250 ppm on root growth in lentils. They reported that the increase in cadmium concentration decreased the shoot dry weight, the amount of proline increased despite the decrease in seedling growth, and an increase was achieved in both roots and shoots. They reported that all Zn concentrations decreased the phenolic concentration of the sprouts in the seedling.

Researchers (Mokhtar et al., 2013) investigated the effects of phosphorus (0, 50 and 100 kg TSP ha⁻¹) and zinc (0, 10 and 20 kg ZnSO₄ ha⁻¹) fertilization on yield and yield components of lentils in Iran in 2011-2012. in their research; They found that the effect of phosphorus on plant height, number of pods per plant, 1000 seed weight, seed yield and biological yield were significant. At the same time, they reported that the effect of zinc application in the soil on plant height, number of pods per plant, 1000-seed weight and seed yield were significant.

Rahman et al. (2013) conducted their research to evaluate the effect of nitrogen applications (13, 19 and 25 kg ha⁻¹) on the agro-physiological properties of 3 lentil cultivars in Pakistan; reported that different varieties and nitrogen ratios have a significant effect on the growth and development of lentils. They reported that the highest seed yield was 976 kg ha⁻¹, the maximum number of pods per plant was 47.29, the thousand-seed weight was 21.89 g, the biological yield was 3954.5 kg

ha⁻¹, and the highest grain yield was obtained from the application of 25 kg nitrogen per hectare.

Researchers (Singh and Bhatt, 2013) reported that zinc levels [Zn₁ control (0.0%), Zn₂ (0.02%), Zn₃ (0.04%), Zn₄ (0.08%)] in late sown lentils in mixed planting and single planting system in 2009 and 2010. In their research, in which they examine the effects; They determined that the effect of application to the leaves twice, the first before flowering and the second after the pod, on the number of pods, 1000 seed weight, seed yield and biological yield. They reported that the effect of zinc application in the soil on plant height, number of pods per plant, 1000 seed weight and seed yield were significant.

The researcher (Upadhyay, 2013) examined the effect of sulfur and zinc on the yield and quality of lentils in alluvial soils; It was reported that 30 kg of sulfur per hectare increased the grain and straw yield by 34.8% and 28.4% on average compared to the control, and 4 kg of zinc per hectare increased the straw and grain yield at almost the same rate.

Sadeghi and Noorhosseini (2014) investigated the effects of zinc, iron and iron + zinc foliar fertilization on yield and yield characteristics of lentils in Dylaman in 2012 and 2013 in Iran. As a result of the study, they reported that the application of iron + zinc and iron alone increased the yield by 37.71% and 27.12% compared to the control. They reported that the interaction of fertilizer and fertilizer application time and the application of Fe + Zn during the leaf and flowering period increased the number of seeds per pod (2.28) to the maximum. They stated that

the maximum 1000 grain weight was obtained when the leaf surfaces were sprayed twice (10-leaf and flowering period).

In a study investigating the effects of zinc levels in lentils [Zn_1 control (0.0%), Zn_2 (0.02%), Zn_3 (0.04%), Zn_4 (0.08%)] (Singh and Bhatt, 2015), the maximum grain yield was 1238.6 kg ha⁻¹ 0.04%. It has been reported that the lowest 1063.1 kg ha⁻¹ from zinc application was obtained from the control group. They reported that the grain yield increased by 16.2% by applying 0.04% zinc leaf fertilization before flowering and after pods in late sowing conditions.

CONCLUSION

It is well known that it is necessary to increase plant and animal production in order to meet the food requirement of the increasing population. In particular, legumes are very important plants in eliminating the protein deficit. However, often insufficient and/or unbalanced fertilization and unfavorable soil conditions significantly reduce crop yields. Insufficient levels of nutrients available to plants in soils is one of the most important problems encountered in aquaculture in recent years.

Although productivity in agricultural lands is inevitable; It is extremely important that the water, soil and air, which constitute the living environment of the society and future generations, are not polluted. For this purpose; Organic matter content of existing soils should be increased or at least the current situation should be maintained in order to obtain high yields by preserving the quality and vitality of the soil with a harmless method instead of methods and tools that have harmful

effects on the environment in agricultural production. The quality of herbal products grown in soils with good organic matter content is also good (Alpates, 2014; Güneysan, 2014; Tarım Library, 2014).

Human health and environmental problems are important issues that all countries of the world focus on today. In the face of the increasingly negative effects of agricultural products and conventional agriculture, the transition to organic agriculture has gained great importance. Organic farming is an alternative farming method for environmental protection, which can prevent agricultural environmental pollution and eliminate the negative effects of chemicals on humans (Yolcu, 2013). One of the biggest factors that cause environmental pollution and deterioration of the natural balance is agricultural practices where chemicals are used intensively. Agricultural practices other than organic agriculture, in which artificial fertilizers and pesticides are used at an excessive level, not only cause environmental pollution and deterioration of the natural balance but also threaten the lives of all living things by reaching the food chain (Aksoy, 2001). Heavy metals, which can be found in soil, water and air at different rates, cause pollution above a certain concentration. The widespread accumulation of heavy metals in the environment poses an increasing danger to all living things. All elements that pollute the environment cause stress in plants. Stress, on the other hand, affects the physiology of plants, changes their genetic potential, restricts their productivity, and leads to their death, resulting in large proportions of crop losses (Munzuroğlu and Zengin, 2004). In this respect, edible legumes; It is an essential

plant group for alternation systems. Edible legumes are valuable for our nutrition and show a significant superiority over other food groups in terms of high protein content in their dry grains.

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CHAPTER 10

EFFICIENT BIOLOGICAL NITROGEN FIXATION UNDER STRESS ENVIRONMENT

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INTRODUCTION

Agriculture has been challenged for many decades and during the 21st century too as a result of the increasing population and the increasing demand for natural resources in the world (Giljum et al., 2015). The demand for various agricultural products results not only in increasing the arable land causing deterioration of biodiverse ecosystems but also increase chemical fertilizers consumption (FAO, 2015). In order to meet the sustainability in global food demand, numerous mitigation approaches are required to decrease their harmful effects on the environment (Pikaar et al., 2018).

Adoption of better fertilization policies like good quality fertilizers, plant stress reduction and reduction of environmental influences like greenhouse gas release on the production of different crops will help in mitigation strategies. A traditional approach to increasing the yield of crops to the maximum level can be achieved by the application of inorganic fertilizers containing all necessary nutrients which are readily available to the plants but the remaining quantity of these inorganic nutrients either accumulate in the soil or leaches out into ground-water (Steiner et al., 2007). Soil organic carbon (SOC) depletion as well as its rapid availability can be avoided by the effective management of nitrogen (N) an essential element of agricultural sustainability in the soil environment. This usually involves the process of biological nitrogen fixation (BNF) using microbes because nitrogen in this form is directly absorbed by the plants, and causes less volatilization, denitrification and leaching. About 80.0% of BNF come from

symbiotic relationship between roots of legume plants and various *Rhizobium* ssp. *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium* and *Mesorhizobium*. Farmers can also use various organic fertilizers naturally made from plant- or animal-based materials such as soybean meal, feather meal, or blood meal (Diacono and Montemurro, 2011). The degree of decomposition and nutrient discharge is based on the microbial action of soil microbes, normally known for the slow release of such nutrients. However, the use of traditional organic fertilizers along with the soil improvements can lead to the addition of heavy metals (HM) like zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd) (Diacono and Montemurro, 2011). In addition to stresses on plants, heavy metal causes negative effects on the environment and potentially be detrimental to human as well as animal health; accordingly, a novel moderation approach should be implemented. Thus, decreasing the effect of agriculture on environment, reducing the direct (field) as well as indirect (fertilizer production) effects are necessary and intensely related to nutrient usage efficacy and crop yield.

Microbial activities and their biomass in the rhizosphere can have many benefits such as nitrogen fixation, phosphorus solubilization and bio-availability, deposition of organic matter, effect on the environment and stress management. A benefit of microbial usage compared to traditional organic fertilization is their concerns, production, and can be manufactured using recycled resources (Verstraete et al., 2016; Pikaar et al., 2018). Microbes contain a high level of N₂ content, which can be

gradually released and ultimately help in soil fertilization (Pikaar et al., 2018). Phototrophic microbes promote plant growth by the production and release of phytohormones and vitamins (Rana et al., 2016). Biological nitrogen fixation was the primary source of converting atmospheric nitrogen into a usable organic form until the Haber–Bosch process was discovered in the early 20th century and commercialized. Fueled by an unlimited supply of hydrocarbons, being a chemical process provided an easily accessible source of N fertilizers, which played a central role in the crop productivity revolution. Nitrogen fertilizer use in cereal and other crops became a routine part of the agricultural practices, and farmers were even tempted to supplement the legume crops with nitrogen. Undoubtedly, N fertilizers were paramount in providing a food security cover which otherwise would have been difficult to increase productivity level (Hawkesford et al., 2012).

The intensive use of inorganic fertilizers is an environmental concern. A high carbon footprint and nitrogen pollution of water bodies such as eutrophication provided enough reasons to pause and question the sustainability of the steep upward curve of N fertilizer usage. Due to the environmental concerns regarding the inorganic fertilizers and the additional cost of cultivation, the significance of BNF has re-gained focus in agriculture production system. Nitrogen is an important major nutrient for achieving the higher agricultural productivity goals to feed the growing population. Improvements in BNF can play an important role in bridging the supply/ demand gap and reducing dependence on chemical fertilizers. There are many areas where improvements can be

made to increase the output of fixed nitrogen. The efficiency, as well as efficacy of the Rhizobia, could play an important role in determining the output. The former relates to the catalytic efficiency of nitrogenase using the host nutrients, whereas efficacy indicates the performance of rhizobia under given conditions (Philippot and Hallin, 2011). The performance of rhizobia depends upon several factors of environmental or soil origin which can adversely affect its survival and performance with negative consequences on nitrogen fixation. Extensive efforts have been made to search for microorganisms with improved efficiency of BNF, are adapted to the extremes of environments, and are more competitive in a rhizosphere. The identification of such strains of microbes responsible for BNF which are specific to the particular conditions provides the opportunities to study the adaptive mechanisms.

1. RHIZOBIAL SYMBIOSIS

Nitrogen is a macronutrient normally available to the plants by mineralization of organic matter in the soil (Hawkesford et al., 2012). In order to ensure the healthy growth of plants, adequate nitrogen levels can be supplied through synthetic fertilizers, but its application is expensive as large fossil energy is required for their production. Furthermore, these inorganic fertilizers are prone to leaching and may contaminate the ground water too. Few soil-bacteria known as Rhizobia establishes a symbiotic relationship with legumes to fix atmospheric nitrogen and convert it to an available form by the process of assimilation for plants. In exchange, the legumes provide them

carbohydrates manufactured by photosynthesis. The process of biological nitrogen fixation (BNF) can decrease the usage of inorganic nitrogenous fertilizers, and ultimately reduces environmental pollution, and help in sustainable agriculture (Oldroyd, 2013). Rhizobia survive either in the soil as free-living bacteria or inside root nodules of legume hosts. In the root nodules, rhizobia translate atmospheric di-nitrogen (N_2) into ammonia with the help of nitrogenase enzyme in an ATP-dependent manner. The nitrogen in the form of ammonia can be assimilated by the host plants responsible for enhanced plant growth and production (Perret et al., 2000). The pairing between rhizobial strains and its host plant takes place following a composite molecular cross-talk that often involves cell to cell communiqué and definite host specificity. The bacterial entry into root cells needs a suitable recognition of a particular chemical signal by the rhizobial Nod-factor which is a signaling molecule of the host plant. The recognition of such molecules causes the curling of root hairs which allow the entry of bacteria into host plant (Oldroyd, 2013). After rhizobial entry, it produces an infection thread by an invagination of plant membrane at the site of infection on root hair. The bacterial entry takes place through these deformed root hairs into the host cells and grows down to cortical cell layers into nodule meristem. Under certain circumstances, the bacterial entry into roots takes place by crack entry i.e. through cracks on root cells (Sprenst, 2009). Rhizobial entry into roots also takes place through the inter-cellular spaces of epidermal cells. An effective infection process finishes with the formation of the nodule (Oldroyd, 2013) that begins with the re-initiation of cell division in cortical cells

of roots allocating rhizobial cells, and initiates nitrogen fixation in exchange of carbon from legumes (Oldroyd, 2013). Rhizobia have currently comprised of 14 different genera and most of them are agriculturally important nitrogen-fixing genera belonging to class *Alphaproteobacteria*. Only a few genera belong to the class *Betaproteobacteria* (Moulin et al., 2014). Currently, more than 89 complete genome sequences are available from the rhizobia including different strains and symbiovars. The size of rhizobial genomes ranges between 6.5-9.0 Mb including plasmids more than 2.0 Mb (Alexandre and Oliveira, 2016). Rhizobial genomes are comprised of two major components: one core genome (higher GC content) that includes housekeeping genes which are responsible for the functioning of cells and other genes involved in its maintenance, and second is the accessory genome, which is located on the plasmid or chromosomal islands (lower GC content) and is composed of genes that confer special characteristics to these microorganisms such as antibiotics resistance and symbiosis genes (Laranjo et al., 2014). The two main groups of genes that are responsible for the symbiosis process in rhizobia are the genes involved in the nodulation process, and those responsible for nitrogen fixation. Nodulation genes (*nodABC*) encodes enzymes responsible for biosynthesis and secretion of Nod-factors, which include lipo-chito-oligosaccharides (LCOs) that interact with plant flavonoids, and are essential for determining the *Rhizobium*-legume interaction (Oldroyd, 2013).

Rhizobium spp. can have different *nod*-genes and therefore, can yield LCOs with different structures (Limpens et al., 2015). The genes involved in BNF process encode nitrogenase enzyme (*nifHDK*) and are accountable to capture and convert atmospheric nitrogen into ammonia. In addition, several other genes are essential in nodule formation as well as nitrogen fixation. For example, *noe*, *nodX*, *nodPQ* and *nodeF* genes are involved in the synthesis of *Nod*-factor substituents, while the genes *nifA*, *fixLJ* and *fixK* encode transcriptional regulators, and *fixABCX* is involved in the electron transport chain to nitrogenase (Laranjo et al., 2014). Besides the genes accountable for symbiosis and crucial for interaction with legume, Rhizobial genomes carry other genes essential for its lifestyle like stress response genes that help the bacteria to survive under unfavorable conditions in soil as well as inside the root nodules.

2. GENES RESPONSIBLE FOR STRESS

Bacteria not only colonize under stress conditions but also survive within a range of varied host plants. Irrespective of a specific environmental condition, bacterial strains are least affected by unfavorable conditions. The major factors responsible for bacterial stresses are temperatures, salts, pH and nutrient deficiency. Most of the genes playing in these stresses are conserved across the bacterial species taking into account different environmental niches in which bacteria can survive. To survive in stressed environments needs an ability to feel such changes respective environments and therefore, modulate gene-expression for the adjustment of microbial physiology

to a new environment. A rapid and efficient way of modulating gene expression is the regulation of a particular transcriptional regulator class known as sigma factors. These sigma factors are needed for transcription initiation which allows differential gene expression by targeting the RNA polymerase to specific promoters (Feklistov et al., 2014). For example, in *Escherichia coli*, σ_{70} (RpoD) is housekeeping sigma factor, whereas σ_{32} (RpoH) regulates the heat shock response. Analysis of bacterial response to a particular stressor allows the identification of gene(s) that are differentially regulated in response to that particular stimulus. Global response analyses like transcriptomics or proteomics studies, provide an important overall map of the alterations at the transcriptional and translational level, respectively.

The heat-shock reaction is well understood in a number of bacteria including Rhizobia (Schumann, 2016). The heat-shock proteins (HSPs) were encoded by some genes which were induced only after an unexpected rise in temperatures. The HSPs comprised of two major classes are involved to defend the host cells from protein denaturation (chaperones and proteases) because of increasing temperature. Chaperones (GroESL and DnaKJ) have an essential function to save mis-folded proteins and allow their re-folding into a functional and native conformation. Proteases (FtsH and ClpXP) are involved in degradation of protein aggregates (mis-folded proteins which are not able to acquire their native conformation). It is thought that these HSPs are frequently involved in response to other stresses, but chaperones and proteases are essential under normal conditions.

3. MOLECULAR BASIS OF THE RHIZOBIAL RESPONSE TO STRESSES

The study on the molecular basis of stress reaction in rhizobial strains is very fascinating due to their exposure not only to different soils but also to symbiotic lifestyles inside the plant root/shoot nodules. The identification and selection of efficient bacterial strains with stress tolerance are important and can be used for field inoculations. When the bacterial inoculum will not survive under drought conditions, its efficacious enactment under the *in-vivo* conditions is significantly not achieved. Transcriptomics has given an important contribution in understanding the global response of these bacteria comprised of the large genome to stress conditions. Different genera of rhizobia nodulating various hosts have been studied and a common trend can be seen, and the transcriptional profile of the responses to the same stressor is found to be diverse among the rhizobia.

Soil salinity is an important problem worldwide, particularly in developed countries where flood irrigation is a common agricultural practice (Ladeiro, 2012). Different legume species can be grown under moderate salinity conditions and effectively increase the available nitrogen (Bruning et al., 2015) however, the ability to establish nitrogen-fixing symbiosis depends on the tolerance of compatible rhizobia to particular stress. The response to the salt shock of *Mesorhizobium japonicum* (Martinez-Hidalgo et al., 2016) while studying the transcriptional response of this strain in comparison to other previously studied rhizobia from different species and hosts

(Laranjo et al., 2014) revealed that the induction of genes involved in the synthesis of osmo-protectant molecules such as trehalose (Lopez-Leal et al., 2014), and *M. japonicum* response to salinity did not include any changes in the transcription of these genes. In addition, no sigma factor was transcriptionally regulated by the salinity in *M. japonicum*, whereas both *Sinorhizobium meliloti* and *Rhizobium etli* showed the up-regulation of *rpoH2* among others. In terms of heat shock response as in case of other bacteria, different rhizobial species showed the induction of genes encoding chaperones and other HSPs (Alexandre A and Oliveira S 2016). It was also shown in the *M. japonicum* that a large proportion of differentially expressed genes was under-expressed following heat shock (Alexandre A and Oliveira S 2014), while in *S. meliloti* and *R. etli*, the number of genes over- and under-expressed was approximately the same (Lopez-Leal et al., 2014). Another study with *S. meliloti* also showed few more genes under-expressed in response to heat shock, but was overexpressed earlier (Sauviac et al., 2007), yet in a much lower proportion than in the case of *M. japonicum*. Global response to acidic pH remains less studied, but the available literature indicates that different rhizobia may show some similarities in their acid transcriptional profile (Lopez-Leal et al., 2014). While in *S. meliloti*, exposure to acidic pH leads to a strong up-regulation of genes involved in exopolysaccharide biosynthesis and a general down-regulation of genes related to the motility and chemotaxis (Hellweg et al., 2009), however in the case of *M. japonicum*, these genes remained mostly unchanged (Lopez-Leal et al., 2014). In both the strains, genes involved in the ABC transporter systems were over-expressed, with the higher

number in case of *M. japonicum*. More recently, a multi-omics approach was carried out to characterize the response to acid stress of *S. meliloti* (Draghi et al., 2016). This comprehensive analysis showed that the acid adaptation requires cell envelope re-modeling that under-control acidic conditions, *S. meliloti* also increases aerobic respiration and alters the central carbon metabolism. The association of ClpB chaperone in symbiosis has been studied and *clpB* mutant strain of *Mesorhizobium* was established symbiotically on the roots of chickpea plants. However, the absence of the ClpB resulted in delayed nodulation (Brigido et al., 2012) signifying its association in symbiosis.

Some gene(s) that were functional in stress reaction was found to be mainly involved in the regulation of heat-shock response and were associated in the symbiotic process e.g. *S. meliloti rpoH1* mutants that have faulty symbiotic phenotypes, therefore displayed a weak establishment and bacterial life and didn't fix atmospheric nitrogen. Whereas, *rpoH2* mutant could show symbiotic phenotypes similar to the wild types. But, *rpoH1-rpoH2* double mutants showed severe symbiotic phenotype than *rpoH1* mutant. Martinez-Salazar et al. (2009) reported that the *R. etli rpoH1* and *rpoH2 rpoH1* mutants showed decreased nitrogenase function, and viability of bacteria in early- and late-symbiosis compared to nodule formation by *rpoH2* mutant and wild type. Despite the fact that efficient experimentation exhibited varied results with *Rhizobium* spp., stress-responsive genes appear to be concerned in root infection by the bacteria and biological nitrogen fixation. Most chaperone mutants showed lesser symbiosis suggesting

the importance of chaperones for rhizobial cells to accomplish an operative symbiosis between legumes and bacteria. The adverse effects due to loss of specific chaperone gene(s) in symbiotic phenotypes are mostly because of the role of these proteins in-folding of newly manufactured polypeptides, re-folding of denatured proteins and disaggregation of proteins involved in the process of symbiosis.

Protein denaturation and aggregation may occur under the environmental conditions found by rhizobia within the host (root) cells such as acidity or microaerobic conditions. Accordingly, the induction of genes encoding molecular chaperones and proteases in the rhizobial cells grown under acidity or microaerobic conditions has been well described by many workers (Lucena et al., 2010). Similarly, both *rpoH* genes were produced under micro-aerobic conditions but the only *rpoH1* was over-expressed in the heat shock and oxidative conditions, whereas *rpoH2* was induced in response to osmotic conditions only. Another regulator *rpoE4* is up-regulated under oxidative, saline and osmotic stress and microaerobic conditions (Martinez-Hidalgo et al., 2016), and it is also over-expressed in aggregated cells during biofilm formation, which is the essential step in the early stages of nodulation. Altogether, the main chaperone gene(s) seems to be associated with the symbiosis of rhizobia-legume interaction. Still, more research in such fields is needed to elaborate the stepwise importance of specific genes in symbiotic associations and other stress responses.

1. RHIZOBIAL IMPROVEMENT USING STRESS-ASSOCIATED GENES

The use of molecular biology in agricultural and allied sciences is developing more interest over the recent years as one of the supportable approaches for enhancing production and productivity (Glick, 2012). Plant growth promoting bacteria (PGPB) which also includes the rhizobia can be utilized as tools to enhance crop yields, while decreasing the negative effects of chemical fertilizers and/ or pesticides on the environment as well as human health. Rhizobial inoculants should effectively fix the atmospheric nitrogen, survive in the soil and compete with local population, and should adjust to environmental situations for a positive and efficient symbiosis. Molecular biology can help to identify and improve bacterial strains, especially genetic engineering to over-express certain specialized genes associated with the symbiosis to improve the bacterial efficacy like nodulation, effective-ness, competitive-ness, stress tolerance, etc. Many genes associated with the stress reaction were over-expressed for improved symbiotic function in rhizobia especially under drought conditions like drought, salinity, oxidative, heat, or biotic stresses.

Over-expression of gene(s) involved in bacterial protection from salinity has improved bacterial strain under such stresses. A strain of *S. meliloti* over-expressing *betS* gene showed better nitrogen fixation in salinized alfalfa plants compared to wild-types. The *otsA* gene encoding an enzyme trehalose-6-phosphate synthase is involved in the biosynthesis of trehalose (Moussaid et al 2015) and over-expressed

otsA *Mesorhizobium ciceri* from *S. meliloti* showed increased growth of *otsA*-over-expressing bacterial strain under saline medium. Chickpea plants inoculated with *M. ciceri* carrying extra *otsA* copies produced a higher number of nodules in roots and also accrued better biomass of shoots when grown in presence of NaCl than the wild-type plants. *P. vulgaris* inoculated with *R. etli* over-expressing *otsA* also showed more nodulation with higher nitrogenase activity and biomass compared to the wild-type. Plants inoculated with *otsA*-over-expressing strain recovered fully from drought stress. Several enzymes involved in the synthesis of plant hormones may also play an important role in improving the symbiotic performance of rhizobia under different types of biotic and abiotic stresses. For example, 1-aminocyclopropane-1-carboxylate (ACC) deaminase is encoded by the *acdS* gene, which regulates the ethylene precursor ACC levels. Plants produce ethylene in response to several environmental stresses and can negatively affect nodulation (Tamimi and Timko, 2003). *M. ciceri* strains (both salt-sensitive and salt-tolerant) transformed with an exogenous *acdS* gene induced chickpea growth significantly higher than the wild-type strain in presence of salt. The *acdS*-transformed salt-sensitive strain was also able to induce nodules to the same extent as that of salt-tolerant strain under salinity (Brigido et al., 2013). Kong et al. (2015) showed the symbiotic performance of *S. meliloti* strain overproducing ACC deaminase in *Medicago lupulina* under the copper stress. Plants inoculated with the *acdS*-transformed strain showed the higher dry weight and total copper uptake but lower levels of copper translocation to aerial parts compared to plants inoculated with the wild-type strain.

The *acdS* gene was also used by (Nascimento et al. 2012) to transform the *M. ciceri* strain and inoculated in chickpea plants grown in non-sterilized soil with the biotic and abiotic constraints for plant growth. The modified *M. ciceri* strain showed an increased nodulation performance and also augmented the total biomass of chickpea plants with reduced susceptibility to chickpea root rot disease (Nascimento et al. 2012). Similar results were obtained for chickpea plants inoculated with the same strain under waterlogging conditions Nascimento et al. 2012. Flavodoxin genes have been used to improve rhizobial performance particularly under the oxidative stress. The overexpression of flavodoxin gene in *S. meliloti* strain was able to protect free-living *S. meliloti* from cadmium toxicity, and had a positive effect on nitrogen fixation of alfalfa plants under cadmium stress (Bianco and Defez, 2010). In addition, Redondo et. al. (2009) observed that alfalfa plants inoculated with rhizobia over-expressing flavodoxin showed a delay in nodule senescence. It is known that the oxygen irreversibly inactivates the rhizobial nitrogenase enzyme. As a higher amount of oxygen is necessary to supply the energy demands of nitrogen reduction process, bacterium produces a high-affinity cytochrome *cbb3*-type oxidase to cope with low oxygen concentration in the nodule (Talbi et al., 2012). The inoculation of *P. vulgaris* plants with *R. etli* strain having enhanced expression of *cbb3* oxidase reduced the sensitivity of *P. vulgaris*-*R. etli* symbiosis to drought (Talbi et al. 2012). The initial effective development of the *Rhizobium* spp. with a chaperone gene was obtained by using a chickpea specific *M. mediterraneum* UPM-Ca36T modified strain with additional copies of *clpB* gene (Paço, et. al., 2016). The

resulting plants showed better nodule growth and more nodule number per plant when inoculated with *clpB*-transformed strain. It was also found that the effectiveness of *clpB*-over-producing strain increased ~60.0% at pH 5 and ~83.0% at pH 7 compared to wild-type strain.

2. REGULATION OF NITROGEN FIXATION (NF) IN NODULES UNDER DROUGHT CONDITIONS

Inside the nodules, nitrogen fixation (NF) is being carried out by the prokaryotic bacteria inside the specific structures known as symbiosomes. The foremost metabolic features regulating the nitrogen fixation must not be dissimilar from extensively studied ones in the nitrogenase of diazotrophic free-living microbes such as (a) sufficient oxygen balance, (b) existence of an energy-producing substrates, and (c) maintaining satisfactory nitrogen status since the nitrogenase enzyme action is extremely depending upon energy.

5.1 Role of the Oxygen

Oxygen plays a serious role in nitrogen fixation (NF) since most of the nitrogenase enzymes are very sensitive to oxygen being irrevocably inhibited in a very short time (Hill, 1988). Nevertheless, NF needs a huge amount of energy, and shoots of the plant may afford the additional carbon through the phloem, but it will not be able to provide adequate energy for the nitrogen fixation under anaerobic metabolic conditions. This condition is determined by the occurrence of leghemoglobin which supports a very low amount of oxygen inside the infested nodule area (~50–100 nM) but provides a high level of delivery

instabilities. Such micro-aerobic circumstances are also possible due to the production of higher affinity bacterial cytochromes. Tjepkema and Yocum (1974) reported a flexible oxygen diffusion barrier (ODB) in the root nodule. The functional response of the root nodules to most of the ecological limitations comprising of dark-ness, heat-stress, defoliation, nitrate supply, deficiency of phosphates, salts and droughts, have been found associated with the function of ODB such as closing of ODB signifies a reduction of available oxygen to the bacterium, and thus results in deficiency of energy for the sustenance of nitrogen fixation. Definitely, the diminished availability of oxygen is perhaps a common stress reaction in nodulating roots of legumes being one of the major factors inhibiting nitrogen fixation. Though, maximum decrease in nitrogen fixation under drought stress couldn't be reinstated by enhancing the exterior supply of oxygen indicating that some other issues might be associated with the water stressed plant nodules, in addition to oxygen deficiency (Diaz et al., 2005).

5.2 Carbon Metabolism in the Root Nodules

Nitrogen fixation depends upon the sucrose supplied by the phloem in many plants. Sucrose is hydrolyzed either by enzymes alkaline invertase (AI) or sucrose synthase (SS), and following the glycolysis pathway, provides the carbon and energy carcasses for respiration and assimilation of ammonia by the bacteria. A decrease in enzymatic activities of SS subsequent to drought, promoting the glycolysis failure in root nodules of soybean (Gonzalez *et al.*, 1995). Similar results were also obtained using pea mutants comprising of only 10% of SS wild

type activities exhibiting the importance of SS for usual nodule growth and utility due to the reason that the plants having less SS level couldn't fix the nitrogen (Craig et al., 1999). A robust association has been observed between SS nodulation, drought stress, and nitrogen fixation in various legume crops. Moreover, SS reduction in function and quantity caused an intense decline in the nodule malate content, provoking a diminishing bacterial function as well as nitrogen fixation (Galvez et al., 2005). The association of the nodule-carbon-metabolism in different legume lines showing contrasting tolerance to the drought has been well documented and displayed a differential response in the nodule SS behavior in soybean (Ladrera et al., 2007).

5.3 Nitrogen Status and Inhibition of Nitrogen Fixation

The status of nitrogen in plants is responsible for regulation of nitrogen fixation in the nodules. Ureide exporter legumes have allantoate and allantoin as main nitrogenous substances supplied from the nodules, and improve our understanding of the special effects of drought stress on nitrogen fixation. This process is more drought sensitive than the amide exporter legumes of temperate origin, but some chickpea species distributed and retained ureide throughout the stresses like drought (Sinclair and Serraj, 1995; Thavarajah and Ball, 2006). We don't know about the status of nitrogen whether it is based on ureide or nitrogen content in plant shoots (systemic regulation of nitrogen fixation) or directly within the nodules (locally regulation) (Purcell et al., 1998; Ladrera et al., 2007). The last one is proved by the experimentations by

splitting roots, in which only half of the roots showed inhibition in nitrogen fixation under drought conditions (Marino et al., 2007).

3. MICROBIAL DIVERSITY UNDER DROUGHT STRESS

Drought conditions influence the microorganism populations in soil by decreasing water film thickness surrounding the soil particles and No. of water-filled pores and enhancing the salt quantity in soil. Microbial (bacterial) movement and dissemination are affected by the characteristics of the water films. Therefore, alteration in such factors by drought conditions will not only affect the microbial commotion and dissemination but also the assortment of rhizobial populations in the soil (Mnasri et al., 2007). Dispersal of the *Rhizobium leguminosarum* in the loamy-sand and silt-loam soils is greatly affected by preliminary soil moisture content (Postma et al., 1989), and the bacterial movement ceases with the discontinuation of water filled pores in the soil due to the drought conditions. A high level of microbial death was observed in sterilized soil which was immediately dried after microbial inoculations. Moreover, the drought environments decreased the bacterial viability in nearly all the rhizobial species capable to make symbiotic association with legume crops. However, has been observed that the genetic improvement for drought tolerance can be achieved in various rhizobial species like *R. leguminosarum* (Athar, 1998). Few strains of *E. meliloti* have been found tolerant to droughts (Mnasri et al., 2007), and the natural existence of *Rhizobium* species in arid environments also showed drought tolerance under certain cases. Amongst them, *Prosopis* species producing nodules was seen to be

highly drought-tolerant, and ability to acclimate in arid environmental conditions, however, soil type as well as different desiccation rates influence the bacterial growth and survival (Fuhrmann et al., 1986).

Free-living local rhizobia may survive under low water potential conditions up to one month (Fuhrmann et al., 1986), whereas, commercial strains often fail to survive under such conditions. The loss of cultural viability in rhizobia indicates that the temperature, type of medium and pH are essential factors for microbial inoculum to survive under drought conditions. Poor water retention capacity of peat-carrier causes a substantial decline in growth and survival of the rhizobial culture. Roots of plant produce some organic substances which encourage the growth of rhizobia, ensuring its survival under water-limiting conditions. Few studies have shown that certain populations of *R. leguminosarum* can be dominated by the non-symbiotic ones of this species (Segovia et al., 1991).

6.1 High Temperature

Rhizobia belongs to mesophilic group of bacteria that require an optimum temperature of 25-30°C for its growth and development. The optimum temperature requirements of the host plant, as well as the bacterium, are prerequisites for positive interaction. Generally, infection of root hairs and nodule function and structure is negatively affected by higher temperatures (Michiels et al., 1994). However, some bacterial strains belonging to rhizobia isolated from arid environmental conditions can grow at 40°C or even above under exceptional

circumstances (Segovia et al., 1991). Even though bacterial strain adaptation to higher temperatures has been reported but with reduced levels of efficiency to establish symbiotic association, generally owing to plasmid loss.

6.2 Salt Stress

In general, it has been observed that the rhizobia are considered to have salt tolerance than their corresponding host plants (Zahran et al., 2007), and few strains of bacteria are capable to grow at 300–700 mM of NaCl. Osmo-regulation is found to be the key approach used by the microbes especially rhizobia to manage the salt stresses with changed polysaccharide manufacturing upon salt management (Lloret et al., 1998). Under certain circumstances, highly salt tolerant bacteria as free-living cells yield ineffective nodules resulting in the decreased symbiotic effectiveness and/ or lower nitrogen fixation. Curling of the root hairs is inhibited under the salinity situations, and formation of infection thread by the bacteria and its colonization are significantly declined. Salt stresses to nodules involved in nitrogen fixation incite rapid inhibition of nitrogen fixation (Tejera et al., 2006). Cultivar reliant tolerance has been observed in faba-beans, common-bean, chickpea and a number of other *Medicago* spp. (Zahran et al., 2007).

4. IMPROVEMENT OF NITROGEN FIXATION (NF) AND YIELD IN DROUGHT ENVIRONMENTS

7.1 Agronomical/ Cultural Operations

The necessary conditions for optimal growth of host plant are prerequisites must be fulfilled for an efficacious symbiotic establishments, and. Cultural/ agronomical operations have a significant impact on the crop and soil conditions. Tillage practices, cropping systems, selection of crop, sowing time and method, lifesaving irrigations, agro-chemical usage, and application of Rhizobial cultures and their inoculations and frequency significantly influence both crop plants as well as bacterial function in the soil rhizosphere under different cropping systems. Some of the important agronomic/cultural practices which affect the nitrogen fixation under the drought situations are briefly discussed below.

7.1.1 Inoculation of Seed

Sowing of seeds immediately after inoculation is important step. If the inoculated seed material persists near or on the soil superficially or is exposed to scorching/hot weather conditions for longer durations, application of additional inoculum is recommended. Though sowing of inoculated seed/ planting material in dried soils is generally not suggested, slurry (paste-like syrup) is usually recommended for bacterial inoculations to maintain the bacterial culture for 15-20 days. Re-inoculation of soil is also advised under prolonged water stress conditions. Under the unfavorable soil and climatic conditions affecting the rhizobia to survive or delaying seed germination, soil application of

rhizobia resulted in improved nodule formation and enhanced growth and yield compared to seed inoculations in various crops like chickpea (Brockwell et al., 1980). The depth of inoculum placement is also an important factor influencing the benefits of soil inoculation of bacteria (Kyei-Boahen et al., 2002).

7.1.2 Soil Condition Improvement

Drought situations amend the physico-chemical characters of the soil in many ways. The deficiency of soil wetness is responsible for the reduction of bacterial movement. Types of soil, bacterial origin and host plant varieties also affects the inoculum development in a particular soil ecosystem. The bacterial motion is lesser in clay type soils as that of soils with a light texture which is perhaps due to the higher adsorbing capability of clay soil particles. Different types of soil have a variable effect on the multiplication and survival of the *Rhizobium* strains inoculated under moisture stress conditions in chickpea (Issa and Wood, 1995). Therefore, few soil factors such as water holding capacity of soil, moisture receding pattern, etc. may have a high level of influence on the survival and longevity of the *Rhizobium*. Organic matter has several positive influences on the microbial activity, soil fertility and moisture-holding capacity.

The organic content declines under the drought conditions due to the reason that a smaller amount of dry-matter is being produced finally affecting the various activities of microbes as well as buffering ability of the soil. The content of organic matter may be enhanced the addition

of plant residue and also by green manuring resulting to enhance the moistness holding capability of soil, though declining soil temperatures thus influencing bacterial movement in significant manure, but the biological nitrogen fixation may be inhibited by increasing the nitrogen content of soil. Other characters of soil like parent material, soil profile depth, ground-water table, pH of soils, crop history, nutrient status and other agronomic operations also influence the biological nitrogen fixation under drought situations. Organic matter oxidation is enhanced because of the fresh and growing crops repetitively cause an increased level of NO_3 in the soil profile that may also disturb nitrogen fixation (George et al., 1992).

Suitable agronomic practices for managing the soils like zero- and minimum-tillage or alternative methods can help to reduce the soil temperature and also conserve moisture content of soil, thereby, increasing the nitrogen fixation (Hungaria and Vargas, 2000). Therefore, minimum-tillage or no-tillage practice is recommended to improving nitrogen fixation activity. Soil-moisture is considered as one of the important inputs in agriculture under dry conditions having a significant effect on microbial activity as well as crop enactment. Improved soil moisture helps in a greater number of nodules and nodule dry-weight. Rain water can be conserved by the suitable agronomical operations throughout the cropping season. Adequate soil moisture content suitable for seed germination is generally also suitable for ideal microbial movements, bacterial growth, and formation of root nodules. Seed-beds must be favorable to preserve the moisture content. Chickpea

crop in the vertisols has shown better nodulation when sown on flat beds as compared to ridges (Rupela and Saxena, 1987). This was because of the higher losses caused by the evaporation due to more surface-area in the ridges being crucial under limited-moisture conditions. Rain water runoff can be reduced an open field well leveled seedbed, and ensures its conservation too. Contour bunding, conservation furrows, compartmental bunds, vegetative barriers, sub-soiling and vertical mulching, amid others, found every effective in conserving moisture content under *In-situ* in various areas. Moreover, mulches using organic matter decrease moisture losses due to evaporation, further reducing growth of weeds and undesired grasses, fluctuating soil temperatures and improving water retention capacity of the soil.

7.1.3 Availability of The Nutrients

Soils, poor in nutrients, have adverse effects on the growth of plants and hinders nitrogen fixation. Nitrogen status and its available form are one of important factors for plant development especially during early growth stages meaning prior to the beginning of nitrogen fixation. Starting application of nitrogenous fertilizer approximately 10-20kg per hectare is generally used to enhance the growth of host plants at the primary stages. Whereas the nitrogen fertilizers applied at lateral stages of growth may hinder bacterial growth, and nodule -formation and – development, therefore, affecting the biological nitrogen fixation by limiting the energy demand of the nitrogen fixation process. Contrary,

legumes crops also required phosphorus (P) and potassium (K) (Kumar et al., 2003) for better growth of plants including root development.

The use of fertilizers comprised of nitrogen, phosphorus and potassium including micro-nutrients increase the root nodulation, enhance nitrogen fixation and also boost the grain yields of chickpea under rainfed conditions (Singh et al., 1999). Application of other mineral fertilizers like 1.0 kg/ha cobalt chloride, 1.0 kg/ha sodium molybdate and 25.0 kg/ha ZnSO₄ along with the rhizobial inoculations has been found to increase chickpea yields by 41.39 and 28.0%, respectively, over control (check plants). Meanwhile, these nutrients are available in limited amounts under dried situations, therefore, supplementary application of nutrients at or near root-zone increases their available form to crops. Mo application along with the Rhizobial inoculations showed an encouraging reaction in legume crops like peas, chickpea and faba beans under drought conditions (Carranca et al., 1999). Generally, non-availability of nutrients affects nitrogen fixation by decreasing shoot growth of plants and also decreases the rate of photosynthesis lowering the carbon supplementation to root nodules. Deficit phosphorus (P) results in small nodule production directly affecting the enactment of root nodules. Consequently, lower N and P inhibits biological nitrogen fixation but causes more oxygen intake in root nodules per unit of fixed nitrogen (Schulze and Drevon, 2005).

7.1.4 Legume and Non-legume Inter-cropping

Growing legume and non-legume crops are common under inter-cropping systems in tropical agriculture. The efficiency of biological nitrogen fixation in legume crops is improved by growing non-legumes. Plant population density under inter-cropping or mono-cropping systems in faba beans and barley under field conditions significantly affects the biological nitrogen fixation (Danso et al., 1987). It was also reported that up to 96.0% of nitrogen was derived from the air in mixed cultivation of faba bean crop in comparison to 65.0% only without any mixed crop. Apparently, major benefit of growing legumes and cereals under inter-cropping system is fixation of atmospheric N₂ by legumes to increase soil fertility contributing to increase the value of inter-cropping constituents.

7.2 *Rhizobium* Management

The identification and selection of legumes and bacterial strains with various sensitivity levels to soil moisture for the biological nitrogen fixation under various environmental conditions are critical for successful nitrogen fixation (NF). The bacterial strains tolerant to moisture stresses can be obtained from their respective legume hosts. The optimum moisture content for the ideal growth of plants is responsible for the maximum progress of nitrogen-fixing inputs by the legume-bacterial interaction in the soil. Nitrogen fixation under drought situations may be influenced by many factors including diversity in bacterial strain ability. Bacterial isolates able to survive under

unfavorable conditions of moisture shall be capable of fixing a sufficient amount of nitrogen as compared to others and carry out all the physiological processes normally. However, the strain variability to fix the atmospheric N₂ under drought situations has not been studied in detail. There are several reports to indicate the variable response of the *Rhizobium* strains in different pulses. Differential influence of *Rhizobium* strains with significant differences in their survival rate has been reported under the rainfed and dryland conditions of chickpea crop exhibiting variable conditions of moisture stress (Kantar et al., 2003). Inadequate information is accessible which will show the accountability of a specialized bacterial isolate for enhanced plant growth under moisture deficit situations (Serraj et al., 1996). Though, fresh application of Rhizobial culture will substitute the diminishing resource of the bacterium in soil rhizosphere. Fresh application of rhizobia will absolutely help the biological nitrogen fixation process under the declining rhizobial populace due to the reduction of soil moisture content and dried soils under drought situations. Bacterial isolates based on their abilities to survive under the drought weather conditions are selected and used in different legume crops enhanced the nitrogen fixation because of the reasons that the freshly applied Rhizobia shall grow very fast on the roots of host plants and also accelerate the nodulation in roots. Dried conditions shall have a negative impact on plant growth at an asexual stage compared to reproductive stage. Moreover, retrieval from the dried stage to common moistness situations varied with the use of Rhizobia and crop. Selection of an

effective strain of *Rhizobium* spp. and their corresponding hosts shall be useful to enhance the rhizobial reaction (Athar, 1998).

The essential conditions to select an effective *Rhizobium* isolates are their competitive ability against other strains, an efficient nitrogen fixation under different ecosystems, the capability of their multiplication in liquid media and sustain in peat-soil, persistence on seed-pellets, survival in soils, capability to subsist in drought situations, capability to move in soils and multiply, nodulation, should be stable under storage conditions and efficient biological nitrogen fixation under existing soil N₂. Hungria and Vargas (2000) reported that the rhizobia obtained from the acidic soils in the tropics showed a good response under water stress conditions. Advanced molecular techniques may help in genetic mapping of *Rhizobium* spp., developing genetically modified rhizobia and identifying better rhizobial strains using molecular markers. Such techniques might be used for screening, identifying and developing new efficient strains of Rhizobia for use under drought situations, ultimately resulting to enhance nitrogen fixation.

7.3 Plant Breeding Programs

There are several dissimilarities amid legume crop species about the quantity of nitrogen fixation. Legumes differ in the nodulation as well as nitrogen fixation when inoculated by the same bacterial strain under different controlled conditions (Kush and Dadarwal, 1980). A significant variation in various nitrogen fixing characters was observed

in chickpea that may be used for genetic mapping and breeding programs (Rupela, 1992). An important effort has been made to use various genetic approaches for accelerating marker assisted selection (MAS) in chickpea (Mantri et al., 2007). Nearly seven thousand ESTs in chickpea were annotated and are presently accessible at open-source databases Like NCBI, mostly from drought proven chickpea populations. Abiotic-stress reaction was investigated by super-serial analysis of gene-expression in chickpea (Kahl et al., 2007). Mantri et al. (2007) reported several gene transcripts were categorized based on cold, salt and drought environments while studying the transcriptional reaction of genotypes showing tolerance and susceptibility to various abiotic stresses in chickpea based on microarrays. Numerous efforts were made to concentrate on drought tolerance in chickpeas, peas and *M. truncatula* (de-Lorenzo et al., 2007) and the recognized gene(s) were utilized to generate a Legume-Stress-Chip readily used for checking stress tolerant genes in microbes and many genes were selected and transformed to plants showing significance in drought environments. The Alfin1 (transcript regulator) over-expressed in alfalfa plants was shown to be responsible for the regulation of NaCl inducible gene-expression responsible for salt tolerance (Winicov and Bastola, 1999). Likewise, transcriptional factor (WXP1) AP2 stimulated many waxes-related genes when over-expressed in alfalfa, enhanced tolerance to droughts (Zhang et al., 2005). The expression of AtDREB1A against stresses enhanced the ability of transpiration in peanut, pea, soybean and *M. truncatula* under droughts (Bhatnagar-Mathur et al., 2005; Dalmais et al., 2008). The *M. truncatula* genome was found to be

closely related to pea genome suggesting that *Viciae* share extended co-linearity (Gualtieri et al., 2002). The understanding of syntenic areas of appropriate legumes shall help the cross-mapping of genes in legumes as well as other agronomical characters that may help to enhance the nitrogen fixation by identification of better nodule forming hosts in drought situations, and transferring those characters to diverse crop plants.

5. CONCLUSION

All microbes, including rhizobial strains, compete with each other for nutrition in the soil. However, rhizobia are also responsible for atmospheric nitrogen fixation in association with roots of legume host plants, and simultaneously they are also affected by a number of abiotic- and biotic factors. Among them, dry conditions are one of the most detrimental limitations because they affect the soil's biological- and physical features. Droughts not only adversely affect plant growth but also decreases the diversity and growth of free-living rhizobia in soil. Both factors determine the development and functions of an efficient nitrogen fixation association. Normally, free-living rhizobia show better tolerance to their respective host plants, but its results are mainly dependent on the level of tolerance. Advanced studies to enhance the drought-tolerance in *Rhizobium* spp. as well as legume hosts have been reported in many species.

The development, along with the improved understanding of the symbiotic nitrogen fixation process shall assist in the identification of

drought tolerant rhizobial strains for efficient symbiotic associations, and to increase crop yields under drought conditions. Genetic approaches like stress responsive genes, their molecular mechanisms and utilization for improvement of rhizobia, molecular markers may pave ways for effective and efficient breeding programs to breed super-nodulating varieties in different legume crops. Alternatively, agronomic practices such as improvement of soil organic matter content, appropriate method of seed inoculation, soil evaporation, starter dose application of N, prevention/mitigation measures, least-/zero-tillage, mixed-cropping or inter-cropping and appropriate soil nourishment will boost the nitrogen fixation under drought environments. High carbon dioxide may increase biological nitrogen fixation and also the available nitrogen in soil, though it might be related to greater phosphorus requirement. The improvement in our understanding of different impacts of dried conditions on nitrogen fixation is essential to enhance the crops yields and quality of produce in legumes under water deficit situations, which may need the scientific know-how for data integration in multi-disciplinary approach. However, it is only possible with dedicated efforts from all stakeholders, particularly the various funding agencies at national, regional and international levels. Moreover, there should be more integration of common goals among these agencies, in order to create an appropriate environment for effective research in this crucial area of agriculture, where sustainable food and feed stocks should be enhanced.

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CHAPTER 11

WEED DYNAMICS AND CONTROL IN LEGUMES UNDER CHANGING CLIMATE

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INTRODUCTION

Legumes are effective contributors to soil health through their nitrogen fixation potential leading to improved crop yields (Esfahani et al., 2016). Further, the role of legumes in global food security is of immense importance. However, productivity of legumes is severely hampered by several biotic factors including those of weeds.

Recently, the climatic changes particularly global warming and rise in atmospheric carbon dioxide concentrations are seriously affecting the crop production and pest management (Jabran et al., 2020). Climate change is likely to impact the growth and control of weeds (Jabran et al., 2015; Jabran and Dogan, 2018,2020). Climate change has both the positive and negative sides for prevalence and control of weeds in legumes. For example, temperature changes may lead to floral composition shifts in various agroecosystems in high altitude and latitude environments, and this weed floral change may come in favor of weeds sometimes and in favor of crops other times. Similarly, changes in temperature and humidity may alter weed seed dispersal (Amare, 2016). This chapter highlights the major weeds and weed dynamics of legumes, critical weed interference periods in legumes, impact of changing climate on the weed dynamics and strategies that may be adapted for weed control in legumes under changing climate.

LEGUMES AND WEED DYNAMICS

Weed Dynamics in Chickpea (*Cicer arietinum* L.)

Chickpea is one of the most valuable legume crops because of its rich protein contents (18-30%). The crop plays an important role in human diet (Maghsoudi and Eizadi-Darbandi, 2021). This crop is a weak competitor against weeds due to low height and slow growth and may face 40 to 90% yield reduction. Some of the common weeds of chickpea include *Chenopodium album* L., *Argemone mexicana* L., *Cyperus rotundus* L., *Carthamus oxyacantha* M. Bieb., *Cenchrus ciliaris* L., *Asphodelus tenuifolius* Cav., *Polygonum* spp., *Fumaria* spp., *Lathyrus* spp., *Vicia sativa* L., *Cynodon dactylon* (L.) Pers. and *Cirsium arvense* (L.) Scop. (Khan et al., 2018). *Lolium rigidum* Gaudin is a highly challenging weed in chickpea production in Australia (Mahajan et al., 2019). *Avena fatua* L. and *Artemisia Ludoviciana* Nutt. also cause serious problems in chickpea in Australia. *Avena fatua* and *L. rigidum* originally affect wheat (*Triticum aestivum* L.) production but also have become common weeds of chickpea (GRDC, 2018; Mahajan et al., 2019). Other common broadleaved weeds in Australia which take advantage of the poor competitive ability of chickpea are *Argemone ochroleuca* Sweet, *Emex australis* Steinh., *Silybum marianum* (L.) Gaertn., *Fallopia convolvulus* (L.) Á. Löve, *Polygonum aviculare* L., and *Cyclospermum leptophyllum* (Pers.) Sprague (GRDC, 2018).

Chenopodium album, *C. murale* L., *Fumaria indica* (Hauskn.) Pugsley, *Rumex dentatus* L., *V. sativa* and *A. fatua* were listed as major weeds in Punjab, the region of Pakistan with highest chickpea

production (Abbas et al., 2016). Other important weeds of district Lakki Marwat, NWFP in Pakistan are *Asphodelus tenuifolius* L., *Astraglaus* spp., and *Medicago denticulate* Willd. (Hassan et al., 2010). In Turkey, some of the chickpea (and wheat) weeds are *Veronica hederifolia* L., *C. datcylon* and *Avena sterilis* L. (Üstüner, 2016).

In Iran, chickpea production is faced with the daunting challenge of dealing with various broadleaved weeds including *Sinapis arvensis* L., *Carthamus oxyacanthus* M. Bieb., *Galium tricorntutum* Dandy, *Lepidium draba* L., *Conringia orientalis* (L.) Dumort. and *C. arvensis* (Shahbazi et al., 2019). Meanwhile, *Thlaspi arvense* L., *Anthemis cotula* L., *C. album*, *Salsola tragus* L., *Kochia scoparia* (L.) Schrad. and *Lactuca serriola* L. are the common broadleaved weeds affecting chickpea production in the USA (Singh and Diwakar, 1995; Boydston et al., 2017). Similarly, chickpea production in Mexico, North America is affected by weeds such as *C. arvensis*, *Chenopodium* spp., *Melilotus indica* (L.) All., *Phalaris minor* Retz., and *Polygonum* spp. (Singh and Diwakar, 1995).

Weed Dynamics in Lentil (*Lens culinaris* Medik.)

Lentil is one of the most common legume crops that provide major protein and fiber for human consumption (Yang et al., 2021). Weeds cause a major limitation in lentil growth and yield due to its slow early growth, short stem and low pod positioning (Maghsoudi and Eizadi-Darbandi, 2021). Major weeds of lentil include *S. arvensis*, *Triticum* spp., *Convolvulus arvensis* L., *Vicia* spp., *Avena* spp., *Centaurea depressa* M. Bieb., *V. pyramidata* and *Fumria officinalis* L. (Tepe,

2014; Guncan, 2014; Kraehmer, 2016; Pala et al., 2018). The weeds of lentil seem to differ with location. *V. sativa* is commonly found to grow with lentils in Turkey and India. In a lentil weed survey in Sanliurfa, Turkey by Bukun and Guler (2005) *Galium aparine* L., *Hordeum spontaneum* K. Koch, *L. serriola*, *A. fatua*, *Scandix pecten-veneris* L., *Isatis tinctoria* L., *Salvia spinosa* L., *Orobanche aegyptiaca* Pers., *Matricaria chamomilla* L., and *Vaccaria pyramidata* Medik. were determined as important weeds having importance values of species scores higher than five. *Galium aparine* and *H. spontaneum* were also noted to be in high frequency and density. In addition, *S. arvensis*, *Ranunculus arvensis*, *Cephalaria syriaca* (L.) Schrad. Ex Roem. & Schult., *C. depressa*, *A. sterilis*, *Papaver* spp., *Silene conica* L., and *V. sativa* were reported in Diyarbakir, Turkey (Pala, 2019; Balli and Ozaslan, 2020).

Some common weeds of lentil in India include *C. dactylon*, *C. rotundus*, *Anagallis arvensis* L., *Cannabis sativa* L., *A. fatua*, *A. mexicana*, *C. arvense*, *C. arvensis*, *Fumaria parviflora* Lam., *Parthenium hysterophorus* L., *Lathyrus aphaca* L., *M. indica*, *P. minor*, *Solanum nigrum* L. and *V. sativa* (Mahanta et al., 2003; Kumar et al., 2018).

Weed Dynamics in Faba Bean (*Vicia faba* L.)

Faba bean is a valuable legume species because of its wide range of uses i.e., food, feed and green manuring (Lavania et al., 2015). Weeds are major constraint in faba bean production and their unrestricted growth caused 80% yield reduction (Mohamed et al., 2004). *Echinochloa crus-galli* (L.) P. Beauv., *F. convolvulus*, *Persicaria*

lapathifolia (L.) Delarbre, *C. album*, *Galeopsis tetrahit* L., *S. arvensis*, *Veronica arvensis* L., *Taraxacum officinale* (L.) Weber ex F. H. Wigg., *Sonchus arvensis* L., *Elytrigia repens* (L.) Nevski, *Plantago major* L., *Equisetum arvense* L., and *C. arvense* were reported in high density and frequency in Lithuania (Romaneckas et al., 2021).

In Mexico, some common broad-leaved weeds of faba beans were *P. aviculare*, *Glebionis coronaria* (L.) Cass. Ex Spach, *Vaccaria hispanica* (Mill.) Rauschert, *Papaver rhoeas* L. while *Bromus sterilis* L. and *P. minor* were two common grass weeds (Boutagayout et al., 2020).

Weed Dynamics in Field Pea (*Pisum sativum* L.)

Field pea is a legume that has a high-level protein and amino acids. Weeds are considered as one of the biggest constraints in its production as they cause a huge yield (77.2%) and quality reduction (Singh et al., 2016; Tripathi et al., 2001). In US and Canada, the reported weeds of importance are *A. fatua*, *S. arvense*, *Hordeum vulgare* (Harker et al., 2001) while in New Zealand, these are *Stellaria media* (L.) Vill, *Stachys* spp., *Spergula Arvensis* L., *Achillea millefolium* L., *Chenopodium* spp., *Rumex* spp., *P. aviculare*, *T. officinale*, *Solanum* spp., *Trifolium* spp., *Polygonum convolvulus* L., *G. aparine*, *V. sativa*, *E. repens* and *A. fatua* (Munakamwe and Mckenzie, 2008).

Chenopodium album, *S. arvensis*, *S. media*., *Elymus repens* (L.) Gould and *Trifolium* spp. are similarly found in high frequency among field peas in Finland (Salonen et al., 2005). Other weeds of high frequency

include, *Erysimum cheiranthoides* L., *F. convolvulus*, *F. officinalis* L., *Galium spurium* L., *Galeopsis* spp., *Lapsana communis* L., *Lamium* spp., *P. lapathifolia*, *S. arvensis*, *Tripleurospermum inodorum* (L.) Sch. Bip., and *Viola arvensis* Murray.

Weed Dynamics in Dry Bean (*Phaseolus vulgaris* L.)

Dry bean is a high value legume crop that is grown worldwide. Weed interference can cause a huge yield reduction and it varies according to weed type, weed density and environmental conditions (Harker and O'Donovan, 2013). *Amaranthus retroflexus* L., *C. album*, *Abutilon theophrasti* Medik., *Xanthium strumarium* L., *Polygonum persicaria* L., *S. arvensis* and *Cyperus esculentus* L. are listed as weeds of high frequency in Canada which is a major producer of dry beans (Soltani et al., 2012).

Studies on the effect of weed interference on the growth and yield of dry bean suggested that *C. album* and *A. retroflexus* were dominant weeds while *A. blitoides*, *C. arvensis*, *E. crus-galli*, *C. dactylon*, *S. arvensis*, *Setaria viridis* (L.) P. Beauv., *Malva neglecta* Wallr. and *P. major* are widely found weeds in dry bean field (Ghamari and Ahmadvand, 2012).

Weed Dynamics in Soybean (*Glycine max* (L.) Merr.)

Soybean is an important leguminous crop that is grown worldwide for both edible and fodder purpose. Weeds can cause 37% yield reduction which is much higher as compared to other pathogens and insect pests (Oerke, 2006). *Sorghum halepense* (L.) Pers., *E. crus-galli*, *Ambrosia*

artemisiifolia L., *Conyza canadensis* (L.) Cronq., *C. bonariensis* (L.) Cronq., *Bidens pilosa* L. and *Eleusine indica* (L.) Gaertn. are mostly found weeds of soybean crop in USA, Brazil and Argentina (Vivian et al., 2013). Moechnig et al. (2013) observed that *P. convolvulus*, *A. theophrasti*, *Amaranthus tuberculatus* (Moq.) Sauer, *C. album*, *Artemisia biennis* Willd., *Oenothera biennis* L., *Ulmus americana* L., *Equisetum hyemale* L. and *E. arvense* are important soybean weeds in South Dakota, USA. Increase in weed density (annual weeds) causes a significant yield reduction in soybean (Hazra et al., 2011).

CRITICAL WEED INTERFERENCE PERIOD

Tanveer et al. (2015) described three periods of weed interference as, 1. Period prior weed interference (PPWI) - it is the crop emergence time and weeds may grow with target crops without effect on productivity; 2. Total period of interference prevention (TPIP) - at this time, weed control must be done to ensure maximum crop yield; and 3. Critical period of weed interference (CPWI) - a delicate interval between PPWI and TPIP where lack of weed control or improper application of control methods may lead to yield losses.

Weed interference periods are dependent on a range of factors such as soil characteristics, environmental conditions, weed community, and the target crop hence an understanding of the weed interference periods are important to cost saving on herbicides use, effective weed control and reduced crop losses (Tepe et al., 2011). The onset of the critical weed interference period is determined by the weed density (Tanveer et al., 2015).

Critical weed interference in legumes differ with locations and weed species (Morishita, 2018). For instance, in the Mediterranean region, the critical period of weed interference is 50 to 69 and 28 to 33 days after emergence in chickpeas and faba beans, respectively (Frenda et al., 2013) making weed dynamic control in these legumes important during these periods. Although most of the farmers try to grow chickpea during early winter or autumn season, the suitable times for chickpea growing in Iran are autumn, spring or early winter. Because of a longer growing season in the winter planting, the yields obtained in winter are much higher than the spring planting. Importantly, the autumn planted chickpea crop faces a higher weed competition. Fedoruk et al. (2011) reported the critical weed interference period of soybean, chickpea, and field pea as 14-28 days after sowing.

In the USA, Morishita (2018) stated that the critical weed interference period of dry beans was five weeks to control annual weeds whereas control of *Solanum physalifolium* Rusby should be done in 6 to 9 weeks post emergence. Also, lentils, grown in Washington USA and Saskatchewan, Canada were more competitive against weeds in relation to those from Northeast Jordan (Smitchger et al., 2012; Fedoruk et al., 2011). Among the lentils of Jordan grown under similar conditions, the critical weed interference period is 12 to 14 weeks in Northeast differently from 7 to 8 weeks in Northwest (Morishita, 2018). With regards to seasons, lentils grown in the USA and Canada during the spring were reportedly of equal competitiveness as those grown in Turkey during winter (Tepe et al., 2005).

IMPACT OF CLIMATE CHANGE ON WEED DYNAMICS

Adaptation of weed to diverse range of environment and temperature due to their physiological plasticity in addition to high genetic variation as compared to legumes that have a lower competitive advantage with increased environmental stress indicated by changing climate (Amare, 2016). The impacts of climate change on weed dynamics includes their floral composition and distribution/population density, biomass, competitiveness against other crops, invasion, and management in agricultural systems.

Population Density

In agricultural cropping systems, the density of grown crops is kept constant according to plant population while the density of weed species varies with the degree of infestation and can be determined by richness of soil seed bank (Radosevich et al., 2007). Due to this variation, the competitive ability of weeds increases and in case of legumes, as they are already less competitive so weeds can easily infest in whole field.

Crop-Weed Competition

Competition between weeds and crops are mainly for space, light, water and soil nutrients. This complex crop-weed interaction was reportedly shifted in favor of some C4 weeds (e.g *C. album*) as against C3 weeds (e.g., *A. retroflexus*) with the elevation of CO₂ (Ziska, 2000). In addition, rising temperature reportedly increases competitive advantage of C4 weeds than C3 weeds (Tungate et al., 2007). Piao et al. (2019)

opines that temperature changes in addition to variation in phenology may disrupt food chain in an ecosystem.

Weed Invasion

Increased risk of weed invasion and ability to colonize more environments beyond the native range are likelihoods (Naidu, 2015) with extreme weather conditions which are typical of climate change. Rising CO₂ levels are reported to aid higher weed invasion (Ziska and George, 2004), in addition to the innately high reproducibility of weeds which is a major factor of their invasiveness.

WEED CONTROL IN LEGUMES UNDER CLIMATE CHANGE

The efficacy of weed management is also postulated to be changed along with the change in climate conditions (Jabran et al., 2020). Under climate change scenario, weeds germinate aggressively so more energy and labour-intensive practices are required for managing these weeds (Siddiqui et al., 2022). Both chemical and non-chemical methods are used for controlling weeds under climate change scenario.

Chemical Weed Control

Chemical weed control is an easier, efficient, cost effective and reliable method used for controlling all types (annual, biennial and perennial) of weeds. Under climate change conditions, the chemical properties and effectiveness of herbicide may change (Jabran et al., 2020). A study showed that the effectiveness of an herbicide was changed due to higher weed growth under climate change conditions (Bailey, 2004). Hence, it may demand to increase herbicide dose for obtaining proper weed

control as compared to normal conditions (Bailey, 2004). Change in temperature levels, CO₂ concentration, humidity, rainfall pattern and wind velocities may disturb the physiochemical reactions of herbicides and ultimately change the absorption, translocation and effectiveness into plants (Keikotlhaile, 2011).

Use of high potency herbicide can control the weeds effectively in soybean under climate change conditions (Jinger et al., 2016). For example, use of quizalofop-ethyl 50g/ha can control all grassy weed and chlorimuron-ethyl 6g/ha can control annual grasses, broadleaved weeds and sedges when applied 15-20 days after sowing. Spraying the legumes with paraquat at flowering stage reduced seed setting in weeds (McMurray, 2010).

Climate change also affects the herbicide application pattern i.e., foliar or soil applied by influencing the basic chemistry of applied herbicide. Soil temperature and moisture content can influence the efficacy of soil applied herbicides while atmospheric conditions can influence foliar applied herbicides (Jugulam et al., 2018).

Non-Chemical Weed Control

Following non-chemical methods can be used for effectively controlling the weeds in legume crops under climate change scenario.

1. Crop Rotation

It is considered as the most effective and convenient weed control method under climate change conditions without paying extra costs. Crop rotation encourages diverse weed flora rather than one or few

dominated species that favors diverse control methods of these weeds even under climate change scenario (Liebman et al., 2014). For example, when soybean was rotated with corn or wheat, a diversified weed flora was obtained that can be easily controlled by common control methods (Satorre et al., 2020). A modelling approach showed that mixed legume species increase soil organic matter and soil nutrient while their rotation control weeds and diseases and helps to overcome yield reduction under climate change conditions (Ndufa et al., 2009).

2. Growing of Climate Resilient Crop Varieties

Development and growing of new crop varieties that have potential to combat with climate change and different environmental stresses would be helpful in controlling weeds under climate change scenario (Vollmann et al., 2010).

3. Sowing Time

Early or late sowing of crop cultivars is also helpful in increasing crop competition with weeds. In soybean growing of late maturing cultivars discouraged seed production and weed seed quantity in *Ipomoea lacunosa* L. and *Sesbania exaltata* (Raf.) Cory by enhancement in competitive ability due to maintaining vegetative growth for a longer time (Bennet and Shaw, 2000).

In lentil, increasing seeding density helped in reduction of weed biomass and increasing of yield (Baird et al., 2009). Another study showed that increasing seeding density enhanced the competitive ability of lentils which increased the suppression effect of chemical

control while using in a moderate rate (McDonald et al., 2007). Other practices like crop rotation, tillage and late sowing could also help in improving herbicide efficacy in lentil crop (Dogan et al., 2014; Kayan and Adak, 2006). In grain legumes such as chickpea and lupin, trimming the weed heads that escaped from previous control method, considerably reduced weed seed production (Riethmuller et al., 2009).

CONCLUSION

Climate change and its impacts on crop pests including those of weeds are a reality of today. Use of a combination of chemical and non-chemical weed control will be helpful in controlling weeds under climate change. Breeding of new cultivars with enhanced weeds suppress ability would be a greater contribution towards weed management in various crops under the climate change scenarios. Prediction about the damages caused by weeds in future is also important for achieving sustainable weed management.

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CHAPTER 12

ROLE OF SEED PRIMING IN ABIOTIC STRESS TOLERANCE IN GRAIN LEGUMES

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INTRODUCTION

Grain legumes belong to Fabaceae and are nutritious and low-cost food sources. Legumes have an excellent potential for edible oil and proteins and can play an important role in food security in the present status of global climate change. Except for protein and edible oil sources, grain legumes also are a substantial source of carbohydrates, fiber, isoflavones vitamins and minerals for human and animal life. Also, legumes are a complementary part of crop rotation systems due to symbiotic nitrogen fixation (Rodriguez et al., 2020), strong taproot systems (Ceritoglu et al., 2020a), charming root exudates for soil microorganisms (Sugiyama and Yazaki, 2012), thereby, they provide to improve soil physiological, chemical and biological properties (Gogoi et al., 2018) and increase sequent crop yield (Chimonyo et al., 2019). Worldwide grain legumes occupy 12-15% of arable land to provide 33% of dietary protein and produce 27% of major crop production (Mishra et al., 2014). Environmental factors are the staple indices affecting plant metabolism, growth, yield and crop quality. While desired ecological factors or beneficial organisms promote growth, adverse environmental conditions and harmful organisms can be led to stress in plants. Plant stress is described as physiological, morphological, biochemical and molecular changes depending on abiotic and biotic factors such as drought, salinity, heavy metal, high or low temperature, nutrient deficiency (Willey, 2016).

The yield lost in grain legume cultivation exhibits a fluctuant diagram due to disrupted soil quality and structure by intensive uses of fertilizer

and pesticide, and changing environmental conditions based on climate change (Gabeltova et al., 2020; Paul et al., 2020). Effects of stress factors on plant growth and crop yield can be effective in different growth stages from germination to maturity. Moreover, stress tolerance index and adaptability are changeable phenomenon depending on both species and genotypes. So, it is needed to understand the responses and tolerance mechanisms of grain legume species to different stress factors and how they change with management strategies. There are different methods used for stress management including agronomic traits, nutrient and water management, using of plant growth promoting bacteria (PGPB), seed priming, breeding tolerant line, exogenous applications, bioengineering and omics approaches (Jha et al., 2019; Nadeem et al., 2019; EL Sabagh et al., 2020). Out of these stress management methods, seed priming is a sustainable, easily applicable and cost-effective way to mitigate the adverse effects of different stress factors (Johnson and Puthur, 2021; Sheteiwy et al., 2021).

1. Effects of Environmental Stress on Cultivation and Yield of Grain Legumes

The unexpected factors in environmental conditions by global warming and climate change have been threatening agricultural production, food security and human health. Almost all predictions forecast to rise further 1.7 °C of mean temperatures up to the 22nd century. Besides, the concentration of atmospheric CO₂ had increased at about a rate of 1 ppm/year through the last 50 years and will increasingly continue in the next years (Lu et al., 2019). So, climate change is the precursor of

different environmental stresses and unexpected natural events such as extreme temperatures, drought, erratic precipitation, shifting of seasons.

Drought is basic stress especially grain legumes since their cultivation, especially winter season cultivated, generally bases on rainfed agriculture systems. Even though water deficiency at any stage in the crop cycle from stand establishment to maturity can negatively affect crop growth and reduce grain yield, drought during reproduction and grain filling has more destructive impacts (Pushpavalli et al., 2014). Moreover, when intensive water deficiency is seen the period from pre-flowering to pod filling leads to terminal drought, therefore, it frequently reduces plant biomass, grain productivity, yield and related components (Ghassemi-Golezani et al. 2013). Different researches demonstrated that terminal drought may lead to yield losses about 27-69% in chickpea (Mafakheri et al. 2010), 40-87% in common bean (Martinez et al., 2007; Ghanbari et al., 2013), 29-68% in cowpea (Ghassemi-Golezani and Hosseinzadeh-Mahootchy, 2009; Ahmed and Suliman, 2010), 24-70% in lentil (Allahmoradi et al., 2013; Choukri et al., 2020), 68% in faba bean (Ghassemi-Golezani and Hosseinzadeh-Mahootchy, 2009), 42-57% in pigeon pea (Maleki et al., 2013) and 42-50% in soybean (Kobraee et al., 2011). Although tolerance or sensitivity exhibits significant differences among both species and even genotypes, terminal drought devastatingly affects grain yield in all cases. This devastating reduction in grain yield bases on leaf senescence (Jan et al., 2019), oxidative damage to photosynthesis (Djanaguiraman

et al., 2018), inhibition flowering (Lakzayi et al., 2014), reduction of carbon fixation and assimilate translocation (Zlatev and Lidon, 2012), pollen sterility (Dong et al., 2017) and restricted grain formation and development (Ullah and Farooq, 2021).

Salinity is another vital constraint factor to crop production and, it is considered to increase as a result of different irrigation malpractices and changing environmental conditions. According to the report of the Food and Agriculture Organization of the United Nations (2015) on the status of the World's soil resources, salinity negatively affects over 60 million hectares or about 20% of the irrigated arable area in the world. On the other hand, 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 reduce grain quality (Hoorn et al., 2001; Jha et al., 2019; Ceritoglu et al., 2020b; Özyazıcı and Açıkbaş, 2021).

Extreme temperatures can destructively affect plant growth, grain quality and crop yield in grain legumes depending on species, tolerance and developmental stage. Heat stress during the reproductive and grain-filling period can lead to destructive damages. Based on climatic

requirements, indeed, grain legumes are divided into two groups such as cool season and warm season. While warm-season grain legumes are more tolerant to high temperatures than cool-season ones, cool-season species can tolerate low temperatures (Hall 2001; Sultana et al., 2014). High temperatures than 30-35°C through a few days restrict photosynthesis and electron flow, disturbed metabolic activities (Redden et al. 2014) and damage flower and pod formation, thereby, it leads to diminished grain yield in chickpea (Gaur et al. 2015). Heat stress at post-anthesis inhibits to hold of pollen grains on the stigma and decreases pollen tube growth, which causes the fertilization process leading to ovule abortion or the production of fractured embryos (Farooq et al., 2017a).

Accumulated heavy metal higher than threshold level due to industrial operations into soil leads to dramatic changes in microbial activity, reduces soil fertility and quality, restricts agricultural production (Ahmad et al., 2012). Many studies indicated that cultivation of grain legumes under metal-enriched soils reduce plant growth, grain quality and yield due to nutrient deficiency or direct effects of toxicant matters in green gram (Seneviratne et al., 2017), pea (Haider et al., 2019), lentil (Alam et al., 2019), common bean (Sasi et al., 2019), cowpea (Santos et al., 2018), faba bean (Rafiq et al., 2018) and chickpea (Kumar and Naik, 2020). Higher concentrations of metals have a devastating impact on various physiological processes such as synthesis of chlorophyll pigments (Feng et al. 2010), inhibition of protein synthesis (Brahima et al. 2010), thereby, lead to a sharp reduction in grain yields. Besides,

higher heavy metal concentration in growing medium limits symbiotic nitrogen fixation via damaging legume-rhizobia symbiosis (Fang et al., 2020). All these findings indicate that mitigation and management strategies to environmental stresses in grain legume cultivations occupy a pivotal position.

2. The Mechanism of Seed Priming Technique

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., 2020; Singh et al., 2020). Indeed, the seed priming technique is based on the exposure of seeds to lower external water potential. 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., 2017b; Sita and Kumar, 2020; Ceritoglu and Erman, 2020; Açıkbash and Özyazıcı, 2021a). The priming induces metabolic accomplishments required for radical projection and embryonic growth (Chen et al., 2012). Optimum stand establishment and plant distribution are noteworthy challenges for successful crop cultivation. Seed priming provides faster and uniform stand establishment, early flowering, thereby, better crop yields (Singh et al. 2015; Ullah et al. 2019).

Seed priming promotes the cell cycle by advancing G1 to G2 phase and induces cyclin-dependent protein kinase and DNA polymerase activity. ATP production, mobilization of different storage proteins, lipids and carbohydrates accelerate during priming (Chakraborty and Dwivedi, 2021). Seed priming triggers protein and enzyme synthesis, activates isocitrate lyase and α - and β -amylase, maintains DNA integrity, synthesis new mRNA, increases the energy charge and ATP content, and increases nuclear 4C DNA content, all of which result in cell cycle advancement from the G1 to G2 phase for synchronized germination. The photosynthetic rate increases due to the efficient uptake and assimilation of nutrients and better utilization of photosynthetically active radiation (Xu and Qiu, 2007). Seed priming results in early activation of an antioxidant system that protects membranes, lipids and proteins under abiotic stresses, such as salinity and drought. The higher photosynthetic rate leads to higher carboxylation and better crop growth and development, which ultimately improves crop yield and harvest index (Farooq et al., 2019). Seed priming improves the effectiveness of aquaporins which provides stimulation for the transport of water across the cell membrane. Seed priming leads to cell wall loosening working out faster water uptake and earlier germination process. This physiological process provides a vital advantage in especially drought conditions where seeds slog on for water uptake due to high osmotic resistance (Al-Yasi et al., 2020). Moreover, LEA proteins produced during the priming process preserve cell structure and integrity that result in overcoming various stresses such as drought, salinity, cold, and osmotic stress (Kosova et al., 2007).

Optimum temperature and duration for priming play critical roles since all enzymes activated during seed priming need an optimum and specific temperature to act. Similarly, proper light and aeration during seed priming affect respiration (Nakamura et al., 1982). There are many types of priming methods, some of which are cheaper and some of the others are relatively more expensive. The effectiveness of priming might show differences based on priming material, techniques, application medium and species. The commonly used seed priming techniques are hydropriming (Forti et al., 2020), osmopriming (Ceritoglu and Erman, 2021), halopriming (Tania et al., 2020), solid matrix priming (Sen et al., 2020), hormonal priming (Rouhi and Sepehri, 2020), nanopriming (Pereira et al., 2021), biopriming (Nawaz et al., 2021), thermo-priming (Izadi et al., 2017).

3. Alleviation of Environmental Stress by Seed Priming in Grain Legumes

Seed priming has noteworthy effects to mitigate the destructive impacts of different environmental stresses in legume crops. Osmopriming integrated with biochar improves cowpea growth under salinity conditions via early and synchronized emergence, chlorophyll synthesis, sugar accumulation which induces oxidative stress and Na^+ toxicity (Farooq et al., 2020). Miladinov et al. (2020) stated that hormonal- and halo-priming treatments with KNO_3 (1%), KCl (1%) and Ascorbic acid (100 mg l^{-1}) help to reduce salinity stress in soybean by decreasing free proline concentration, the intensity of lipid peroxidation and Na^+ , and increasing K^+ ions uptake. Moreover, Ascorbic acid

priming leads to an increase in the concentration of vitamin C, while priming KCl and KNO₃ reduced the vitamin C content in soybean seedlings. Seed priming with CaSO₄ and CaCl₂ throughout 4 hours at 25 °C improved the germination characters and seedling growth such as germination percentage, speed of germination, mean germination time, radical length, epicotyl and hypocotyl length compared with control plants under saline conditions (Nabi et al., 2020). Seed priming with brassinosteroid promotes stress tolerance against salinity in soybean and Supplementation of N further enhanced the effectiveness of brassinosteroid in the adaptation of soybean (Soliman et al., 2020). Halopriming treatments reduced salinity stress in chickpea by improving germination characters such as germination percentage mean germination time and uniformity of germination (Khadraji et al., 2020). Seed priming with 2.16 µM benzyladenine enhanced the vegetative and reproductive, and improved physiological responses of soybeans under extreme saline conditions (Mangena, 2020). Sheteiwiy et al. (2021) demonstrated that seed priming with jasmonic acid (JA) improved transpiration rate by 74.85%, net photosynthetic rate by 68.03%, intercellular carbon dioxide concentration by 61.64%, stomatal conductance by 69.88% and total chlorophyll content by 47.41% under saline conditions, respectively. Moreover, JA-priming enhanced abscisic acid, gibberellic acid and JA contents under salt stress compared to control conditions. Açıkbash and Özyazıcı (2021b) reported that increasing salinity noteworthy caused inhibition on germination characteristics in forage pea, however, 6 mM Si-priming through 20 h provided to alleviate salinity stress. Ceritoglu and Erman

(2020) determined that priming of chickpea seeds with 0.2 mM salicylic acid through 12 h at 2 ± 23 °C improved germination percentage, germination index, mean germination time, uniformity of germination and germination rate under saline conditions.

Seed priming with PEG improved agronomic and physiological traits in mung bean and primed seeds exhibited a remarkable resistance to drought stress by enhancing antioxidant enzyme activity such as SOD and POD (Uddin et al., 2021). Zn-priming provides to mitigate the restricting effects of drought stress in faba bean during the early vegetative stage through increasing the total biomass (99.8%), leaf surface area (23%), α -amylase activity (85%), soluble sugar content (54.7%), total chlorophyll content (48.7%), Zn concentration of leaf (79.8%) and decreasing MDA content (42.7%) and antioxidant activities (35.2%) under water deficiency conditions (Farooq et al., 2021). Priming with the extracts of carrot root, garlic cloves, or ascorbic acid mitigated drought stress in faba bean and reduced carotenoids, activities of peroxidase and catalase, ascorbic acid, glucose, MDA and soluble protein contents (Kasim et al., 2017). Seed-priming with ascorbic acid (1 mM) or PEG-6000 (7.8 mM) significantly reduced the detrimental effects of drought stress on seedling growth in alfalfa due to lower accumulations of MDA and H_2O_2 , higher accumulations of total soluble proteins, soluble sugars, proline and total phenolics as well as increased activities of H_2O_2 -scavenging enzymes (Salemi et al., 2019). Abid et al. (2020) reported that harmful effects of drought stress decreased in primed faba bean seeds with 1 mM of β -aminobutyric acid

(BABA). While leaf photosynthesis rate, leaf relative water content, stomatal conductance and transpiration rate increased, water use efficiency decreased depending on priming applications. Besides, BABA-priming decreased H₂O₂ production, MDA and electrolyte leakage levels, cell membrane damage.

CaCl₂-priming improved nodule number and dry weight respectively up to 20% and 34% under 50 µM Cd stress compared with non-stress conditions. Besides, treatment was effective in mitigation the harmful effects of Cd on physiological and biochemical traits of faba bean (Nouairi et al., 2021). Salicylic acid priming enabled increase antioxidant enzyme activity such as ascorbate peroxidase (APX) and superoxide dismutase (SOD) and also reduced MDA content in *Trifolium* seedlings under Al toxicity (Bortolin et al., 2020). When *Vigna radiata* seeds which were primed with salicylic acid exhibited better germination process and seedling growth compared with non-primed seeds under Cd toxicity (Hassan and Mansoor 2017).

Posmyk and Janas (2007) stated that hydropriming is a cost-effective and easily capable strategy for chilling stress in mung bean. Elkoca et al. (2007) stated that PEG-based priming for 24 hours enables faster germination in chickpea under low temperatures. Solid matrix priming with chitosan reduced chilling stress in groundnut by increasing the activities of lipases, levels of gibberellic and indole acetic acid (Rakesh et al., 2017). Mercado and Fernandez (2002) pointed out that solid matrix priming is an effective strategy for cold stress in soybean. Seed priming with Monopotassium phosphate (KH₂PO₄) reduced the

restricting effects of P deficiency in faba bean and provided to increase total biomass, P uptake, and activity of antioxidant systems, therefore, it showed that seed priming might be a sustainable strategy in nutrient-deficient conditions (M'Sehli et al., 2020).

4. Conclusion and Future Prospects

Seed priming with various techniques is an important strategy for environmental stress management in grain legumes. Due to sustainable, eco-friendly, easily capable and cost-effective properties, priming technology has started up with other conventional techniques using to improve stress tolerance. Out of priming techniques, biopriming with PGPR and nanoprimering has been increasingly studied by researchers for last years since they provide versatile gains and relatively new system technologies. Moreover, seed coating technology with PGPR is another encouraging strategy for sustainable agricultural systems although there are still some problems on limited storage time of inoculated seeds.

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CHAPTER 13

**ROLE OF PLANT GROWTH PROMOTION
RHIZOBACTERIA IN LEGUMES TOLERANCE TO ABIOTIC
STRESS**

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1. INTRODUCTION

Plant growth enhancing rhizosphere microorganisms (PGPR) are known to further develop plant execution through different components, for example, creating useful chemicals, upgrading plant healthful status, and decreasing pressure related harm. The connection among plants and PGPR turns out to be especially intriguing in conditions portrayed by imperfect development conditions, like high or low temperature, dry season, soil saltiness, and supplement shortage. As the world's farming faces issues, for example, environmental change and soil debasement, the positive job of PGPR will turn out to be more alluring later on. This part expects to talk about the essential system of the connection among PGPR and plants and will zero in on how PGPR can decrease the abiotic stress harm of beans, which are basic harvests in the human diet. Global horticulture is confronting the overwhelming test of expanding creation and the developing populace size of the showcase. Likewise, the pressing factors of environmental change, land planting, and metropolitan improvement can be utilized to diminish ripe land. These issues are essential. Since they will influence the creation of beans and influence the world, beans are a fundamental piece of society.

To assist with this issue, specialists have directed their concentration toward the investigation between getting plants and plant root frameworks and agribusiness. The examination of plant root framework and soil microbiota affects plant development and advancement, from advantageous to impact. For model, plant development advancement

rhizosphere wellbeing (PGPR) assumes an imperative part in plant development advancement. In any case, it is still ineffectively saw, particularly under natural anxieties like dry season, temperature, saltiness, and degree.

PGPR further develops plant execution through various components, typically synergistically; a few models incorporate delivering plant-development advancing chemicals, further developing plant healthful status, and lessening pressure harm. Moreover, the association among plants and PGPR can further develop plant execution and upgrade protection from biotic and abiotic stresses, which are fundamental attributes of developed harvests.

2. IMPORTANCE OF LEGUMES IN GLOBAL NUTRITION

Beans are yearly plants and have a place with the monocotyledonous Gramineae family. They are a fundamental food hotspot for people since they give almost 50% of the calories burned-through consistently on the planet. What's more, beans are likewise broadly utilized as creature feed, principally for domesticated animals and poultry, and as a crude material for some modern cycles, fundamentally for the creation of hard beverages (Lamb et al. 2005).

In the course of recent years, the expansion in grain creation (+240% is the consequence of an increment in yield for every hectare (+201%), not the land allotted for oat creation Expand (+12%) However, this pattern has as of late debilitated. For instance, the normal yield of heartbeats from 1961 to 2007 was 3.6% each year, and from 2007 to

2017, it dropped to a normal of 2.7% each year. This might be identified with numerous components, including environmental change, soil debasement, the utilization of soil for non-nutritive purposes, agrarian water, supplement and land limitations, and customary rearing limitations (Bini, 2009).

3. ABIOTIC STRESS EFFECTS ON AGRICULTURE

The dirt attributes of most arable land on the planet are imperfect. Any deviation from ideal development conditions will cause a few interrelated responses in the plant, which can be portrayed as adjusting to new natural conditions to keep up with homeostasis. In the event that the pressure endures excessively long or too harshly, it might forever harm plant physiology or cause death (Amundson et al. 2015). Albeit numerous plants can adjust to pressure, the interaction requires energy moved from dynamic development, bringing about more modest plants that adjust to the climate. Abiotic stress, stress brought about by abiotic factors, is viewed as the fundamental driver of worldwide harvest misfortune, and efficiency diminishes by over half every year (Qadir, 2004). Dry season and salt pressure are possible natural perils of agribusiness, particularly in parched and semi-bone-dry regions that are as of now near the restriction of harvest usefulness. Because of an Earth-wide temperature boost and agrarian soil debasement, these regions may presently don't uphold crop planting in the future (Galippe, 1887).

3.1 Climate change

Food security is decidedly associated with financial strength; given that environmental change compromises food creation, this will have wide-going and complex impacts. Since the center of the nineteenth century, the normal temperature has ascended by 0.8°C. Before this present century's over, the climate is relied upon to increment by 1.8 to 4°C from the finish of the last century. This change is causally identified with the ozone depleting substance (like carbon dioxide) created by human exercises, the convergence of which rose from around 284 ppm in 1832 to 397 ppm in 2013 (Samlla et al., 2006)

Despite the fact that CO₂ is for the most part acknowledged as an ozone depleting substance, individuals are presently progressively intrigued by the job of nitrous oxide (N₂O). This compound can be gotten from the denitrification of nitrogen manures regularly utilized in current horticulture. In 2014-2015, the greater part of nitrogen compost was utilized uniquely in cereal crops (Foster, 1986) The renewed introduction of N into nitrogen-helpless soils is a fundamental agrarian practice that has expanded yields in the previous few decades. In any case, the application proficiency of nitrogen manure is low. It is assessed that plants assimilate only 33% of the nitrogen application, and the overabundance nitrogen is lost in surface overflow, penetrates into groundwater, or volatilizes into the atmosphere (Lynch and WHIPPS, 1990). Therefore, despite the fact that N₂O in the environment isn't pretty much as plentiful as CO₂, it is multiple times more viable as an ozone harming substance.

It is normal that environmental change brought about by ozone depleting substance discharges will straightforwardly influence the usefulness of horticultural frameworks in pretty much all aspects of the planet. Albeit numerous rustic destinations in chilly mainland locales will profit with rising temperatures, regions portrayed by mild, tropical dry, or sub-bone-dry environments may confront decreased yields. By mimicking the effect of environmental change on the creation of different beans in various districts of the world, it is anticipated that before this present century's over, heat pressure occasions in Central and East Asia, southern Australia, and focal North America will increment, and southeastern Brazil (rice); northern India, Sahel, South-Eastern Africa, and Central and South America (maize); and Central Asia (wheat). Compass et al. It is assessed that if no actions are taken to decrease ozone depleting substance outflows, a four °C expansion on the planet's normal temperature by 2100 will seriously lessen the food creation of practically all nations world. This will bring about a moderate financial loss of roughly US\$230 million, and agricultural nations in Southeast Asia and Africa are relied upon to confront the main misfortunes (21% and 26% of GDP, respectively) (Walker et al. 2003).

3.2. Agricultural Soil Debasement

Soil debasement is one of the fundamental farming usefulness issues, particularly in tropical and subtropical districts (Lamb et al. 2005). Worldwide, 33% of the land is influenced by some type of corruption. Unseemly agrarian methods, combined with over the top expulsion of

yield buildups and lopsided utilization of synthetic manures, will decrease soil quality, drain natural material saves, and increment disintegration. Besides, eliminating crops from the creation site will bring about the deficiency of fundamental components for plant development. In this way, these components should be ceaselessly once again introduced to stay away from a diminishing in efficiency.

Utilizing soil for rural purposes can prompt debasement of water sources, as corrupted manures can saturate groundwater (Lugtenberg and Dekkers, 1999). As an outcome, numerous waterways in non-industrial nations have serious water contamination and eutrophication issues. In numerous bone-dry and semi-bone-dry districts, water system is a key administration procedure for acquiring adequate usefulness to fulfill food need, yet it can cause unfavorable impacts. Erroneous water system methods have expanded the saline soil, which is currently found in over 20% of flooded land (Philippot et al., 2013).

4. PLANT GROWTH-PROMOTING BACTERIA

A typical confusion in the nineteenth century was that sound plants ought to be sterile and not cooperate with any microorganisms. This speculation was at first addressed by Victor Galippe, who demonstrated that solid plants could have different microorganisms in their tissues. Today, we realize that practically all earthly plants from various conditions communicate with the encompassing microflora at all phases of plant development (Huang, 2019). The connection between plants and microorganisms can be parasitic, advantageous, or cooperative, and can likewise be unbiased or useful to plant

development, and is altogether different because of an assortment of variables (organic and non-natural). PGPR is drawn to plants by the natural emissions delivered through the roots and chooses the root surface and the dirt in direct contact with the seeds. The dirt substrate in direct contact with plant roots is known as the rhizosphere (Samlla et al. 2006), and the external surface of root cells is known as the root surface. Here, colonizing microorganisms can set up supplements and different mixtures with plants (Huang, 2019). Ceritoglu et al. (2021) stated that effectiveness of PGPR inoculation bases on microbial strains and application duration pre-sowing in lentil.

The discharges delivered by plant roots draw in soil microscopic organisms, which can colonize the rhizosphere and plant tissues. Here, they furnish plants with different valuable mixtures to trade supplements, primarily photosynthetic products (DeAngelis et al., 2009). The supplements and natural mixtures delivered from the roots to the rhizosphere come from photosynthesis, and plants discharge up to 30% of photosynthetic items through the roots. These incorporate different classes of mixtures, like carbs, amino acids, natural acids, flavonoids, and lipids, which can be utilized as fuel hotspots for microorganisms. Many soil microbes' insight and dynamic movement to these mixtures are called chemotaxis, which makes microscopic organisms colonize the rhizosphere and root surface (Kawasaki et al., 2016). By creating exudate, plants can pick the sorts of microscopic organisms pulled in by explicit mixtures, in this manner directing the bounty and variety of rhizosphere microorganisms. According to

reports, wild oats can change their rhizosphere bacterial populace, chiefly enhanced in Firmicutes, Actinomycetes, and Proteobacteria. Specifically, the last gathering is for the most part viewed as the fundamental microbial segment in PGPR collaborations due to their fast development capacity and different metabolic pathways that can utilize various exudate compounds as energy sources (Reinhold et al., 1985).

In the model oat plant *Brachypodium*, the rhizosphere microbiome changes approximately bound rhizosphere soil and firmly bound rhizosphere soil and changes in seed roots and nodal roots. It is important that plants can in a roundabout way influence the colonization of the rhizosphere by changing ecological conditions. A few models are pH changes brought about by particle assimilation, lower O₂ and H₂O levels brought about by root breath, and water ingestion (Bouffaud et al., 2012).

Various kinds of root exudates can draw in various PGPRs. For instance, different strains of *Azospirillum brasilense*, a Gram-negative *Alphaproteobacteria*, show fluctuating levels of appreciation for various mixtures delivered by other host plants. Moreover, the creation of root exudates may differ significantly between various plant species (Peiffer et al., 2013). Two further studies, detailed that even other plant species' genotypes could have different bacterial populaces in the rhizosphere. Exudates fluctuate contingent upon various pieces of the root, distinctive formative phases of the plant, or reaction to other development conditions. This implies that a similar plant can

communicate with numerous unfamiliar soil bacterial strains over the long haul and space (El-ZaharHaichar et al., 2008).

Nehletal used the expression "rhizosphere microorganisms" to depict root plane/rhizosphere microbes; however, there are likewise endophytic microscopic organisms that can exist in plant tissues. Up until this point, various communications among plants and rhizosphere/rhizosphere colonizing microscopic organisms have been portrayed, however a few microorganisms are considerably more specific. Once involved in the rhizosphere, they can enter the root tissue and straightforwardly contact apoplast natural mixtures, accordingly keeping away from rivalry with different microorganisms in the rhizosphere. Root infiltration can be dynamic by creating cell divider debasing catalyts, for example, cellulase or inactive, for instance, through breaks framed on the root surface during horizontal root improvement. The colonization past the rhizosphere into apoplasts requires specific microbial morphology. Czaban et al. (2007) describe how the rate of flagellar development in bacterial strains disconnected from the inside root tissue of wheat is multiple times higher than that saw in microorganisms secluded from the rhizosphere. *Bacillus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Serratia* and *Streptomyces* are the most widely recognized endophytic microbes in plant tissues. Bypassing through the endothelial layer, numerous bacterial species can spread from the root, reach and colonize other stem organs. Endophytic microbes can likewise spread from plant tissues to seeds and become the underlying inoculum to colonize posterity plants.

(Companet et al., 2010). The spread of microorganisms between plant ages is a cycle called vertical transmission. Johnston-Monje and Raizada portrayed how current corn assortments and their wild precursors shared the normal endophytic bacterial networks present in seeds. Resulting concentrates on wheat showed how these networks assume a functioning part in plant growth (Nehl et al., 1997).

4.1. Plant Growth Promotion Driven by Rhizobacteria

Galippe's instinct is that plants collaborate with microorganisms for the duration of their life cycle, which has prompted a critical expansion in comprehension of the valuable impacts of microscopic organisms on plant growth (Hallmann et al., 1997). PGPR associations can prompt higher plant biomass, higher healthy benefit, higher endurance rates, and for the most part lower rural sources of info. PGPR centers around beans, which can essentially further develop plant execution in different conditions, particularly those with helpless developing conditions. A portion of the principal benefits acquired by plants are expanded root improvement, in this way expanding protection from temperature and osmotic pressure, soil contaminations, nuisances, and pathogens (Hardoim et al., 2008).

Obviously, the reaction of plants to biotic and abiotic stress requires complex variations of construction and digestion. Moreover, when both biotic and abiotic stresses are applied all the while, the appropriate response of plants is very extraordinary contrasted and the pressure involved alone. Therefore, it is sensible to expect to be that in case plants are presented to biotic and abiotic stresses simultaneously, PGPR

can straight forwardly decrease the effect of biotic pressure by expanding the plant's protection from abiotic stress (Czaban et al., 2007).

4.2. Chemical Related Mechanism

The most completely portrayed system that PGPR can expand grain efficiency is creating different plant development advancing chemicals, which normally influence plant execution in an exceptionally incorporated manner (Dimkpa et al. 2009). Auxin is a sort of chemical that is for the most part blended by the apical bud and moved from that point to different plants. Among these chemicals, the most trademark is indole-3-acidic corrosive (IAA), which upgrades cell stretching and separation, and invigorates sidelong root improvement in roots. Various reports show that auxin creation from PGPR is perhaps the most fundamental instruments of advancing plant development. Barbieri and Galliused two strains of *Azospirillum brasilense* to immunize wheat, one of which was a freak with hindered IAA yield (Compant et al., 2005). They saw how just wild-type strains advance horizontal root improvement. This outcome shows the unmistakable job of IAA in further developing plant root advancement. IAA can by implication work on the healthful status of plants by advancing root improvement (particularly parallel roots) so that plants can investigate a higher extent of soil substrate, which is a fundamental component for getting low-versatility supplements like phosphorus. Gibberellins (GAs) can be delivered by PGPR and are accepted to assume a fundamental part in advancing plant development. These diterpene chemicals are normally

present in plants and direct basic cycles, for example, seed germination, stem extension, leaf development, root development, and root hair abundance (Johnston and Raizada, 2011). One of the most well-known GAs is GA₃, generally called gibberellic corrosive, which assumes an essential part in deciding the source-sink relationship of plants. The job of gibberellin in the reaction of beans to pressure fluctuates with the kind of stress, however as a rule, plants will in general decrease GAs levels when they develop under imperfect conditions. It has been accounted for that exogenous use of gibberellin can work on the presentation of wheat and rice under salt stress and diminish the indications of hefty metal pressure in rice (Herrera et al., 2016).

Numerous PGPRs can corrupt 1-aminocyclopropane-1-carboxylic corrosive (ACC) by ACC deaminase and utilize the debasement items as a nitrogen source. ACC is the biosynthetic forerunner of ethylene. Ethylene is a chemical normally present in plants, and its plenitude for the most part increments with stress. At the ideal level, ethylene is engaged with the basic cycles of tissue separation, root improvement, blooming, grain advancement, and normal tissue senescence and shedding; when over-delivered, it will decrease plant execution. In plants under abiotic stress, ethylene increments can cause chlorosis and organs, particularly early development and senescence of seeds, and hinder root development. By disturbing the ethylene flagging pathway, the collaboration with PGPR can diminish pressure related harm in plants (Lugtenberg and Kamilova, 2009).

Like ethylene, abscisic corrosive (ABA) is a chemical regularly delivered by plants because of different sorts of pressure, particularly osmotic pressing factor [61]. ABA normally partakes in seed and bud lethargy and offers the principal biosynthetic advance with cytokinin. Cytokinin is a plant chemical that normally alienates ABA. In dry or saline soils, responsive oxygen species (ROS) increment the biosynthesis of ABA, which is then shipped to the leaves, where they cause the stomata to near lessen happening and water misfortune. Hence, the dissemination of carbon dioxide to the leaves is diminished, lessening the photosynthetic rate. As per reports, PGPR can build plant protection from salt, decreasing pressure related ABA amassing in plants, and keeping up with photosynthetic efficiency (Trewavas and Malho, 1997).

5. PLANT-BACTERIAL ASSOCIATIONS IMPROVE ABIOTIC STRESS REACTIONS

Microorganisms can noteworthy affect their host plants. PGPR can straight for hardly influence plant development by fixing barometrical N_2 to bioavailable N compounds or delivering development advancing chemicals, and by implication by forestalling the development of plant microbes or expanding plant protection from them. The important condition for microbes to profit plants is rhizosphere limit in light of the fact that the opposition and conditions in the rhizosphere are altogether different from those in huge soils. The rhizosphere contains a higher bacterial bounty than the mass soil, yet the variety is a lot of lower. The plant root framework isn't homogeneous; the thickness of

explicit microbes in various pieces of the root framework is unique, most likely identified with the diverse root exudates delivered from various pieces of the root framework. Another conceivable component for directing rhizosphere colonization is bacterial majority detecting, which manages quality articulation driven by bacterial populace thickness, which can happen inside microscopic organisms of similar species and between various species. Majority detecting influences the intensity of microbes and hence influences root colonization patterns (Glick, 1995).

5.1. Thermic Pressure Variation

Temperature stress prompts changes in the creation of chemicals, particularly ethylene, which normally weakens plant development. High temperature and pressing factor can cause cell protein denaturation and collection. If not controlled, it can prompt cell corruption. The awkwardness among ABA and cytokinin starts from long haul heat pressure during the conceptive stage, prompting grain fetus removal. The warm reaction incorporates restraint of standard record and interpretation and expanded articulation of qualities encoding heat shock proteins and warmth resilience actuated qualities. In actuality, low-temperature stress can upset metabolic cycles, change film properties, cause changes in protein structure, and restrain enzymatic responses. In the event that it happens during sporulation, cold can cause bloom sterility by meddling with meiosis (Barbieri and Galli, 1993).

There is generally little writing on PGPR collaboration with heartbeats at imperfect temperatures, and the system of heartbeat variation isn't distinct. It is recommended that the topographical beginning of microscopic organisms decides the ideal development range for their advantageous connection with plants. In an examination on wheat, it is accounted for that microorganisms confined from cold environments can viably colonize the rhizosphere of plants and increment their protection from low-temperature stress. At the point when wheat plants are vaccinated with microscopic organisms secluded from warm conditions and with stand high-temperature pressure, microorganisms disengaged from various temperatures may endure cold or warm conditions, consequently delivering higher plenitude and colonization in the rhizosphere, outperforming local microbial populaces in competition (Wittenmayer and Merbach, 2005)

At the point when plants are presented to warm treatment, immunization of *Pseudomonas aeruginosa* strains confined from a hot semi-bone-dry climate can further develop the endurance rate, advancement, and biochemical boundaries of sorghum seedlings. Simultaneously, the biomass yield isn't influenced at the ideal temperature. In another investigation, different cold-open minded. *Pseudomonas* species. Vaccinated on low-temperature-developing wheat, and comparable outcomes were acquired. The creator accepts that this helpful impact is identified with the better root advancement of the vaccinated strains, which works on supplement assimilation and in general better transformation to cold.

Since a dangerous atmospheric deviation may altogether change the temperature of most arable land, to keep up with productivity and creation under problematic temperature conditions, it is fundamental to foster beans with upgraded versatility to warmth or cold pressure. Assignment. Albeit further examination is expected to see better the instrument that directs the collaboration of PGPR with plants under such conditions, the investigation done as such far shows how PGPR can be a fundamental wellspring of protection from temperature stress, particularly when they are presented to warm or cold the environment of the trademark region advances relying upon the circumstance.

5.2. Osmotic pressure transformation:

Both dry and saline soil can cause osmotic tension on plants, which can make cells become got dried out because of absence of water (dry season) or absence of water (saltiness). These two pressing factors are as a rule of extraordinary agronomic importance on the grounds that the high saltiness in the dirt is basically brought about by water system, which is important to build creation in numerous spaces of the world with inadequate precipitation. At the point when the water supply is deficient to eliminate the particles in the surface soil, they will amass and expand the saltiness.

Saltiness is likewise a consequence of land clearing since profound underground roots can presently don't keep the water table subterranean level. As the groundwater level ascents, the saltwater that follows will make many square kilometers of farmland crude. Plants developing on this dirt frequently experience the ill effects of osmotic pressure,

decreasing water assimilation and expanding the particle focus in tissues to harmful levels (Parihar et al., 2015; Ceritoglu et al., 2020; Dhiman et al., 2021). PGPR can lighten these pressure manifestations through an assortment of components, for example, the creation of Na⁺-restricting extracellular polysaccharides, the improvement of particle homeostasis, the decrease of ethylene levels in plants through ACC deaminase, and the amalgamation of IAA. The germination pace of wheat seeds immunized with *Pseudomonas* in a saline climate expanded; Egamberdiyeva credited this to the creation of plant development controllers by microscopic organisms (Trewavas and Malho, 1997).

Dry season is viewed as the main source of yield misfortune, which contrarily impacts most physiological cycles of plants. Plant cells react to water misfortune by expanding abscisic corrosive (ABA) creation in the roots, which builds water assimilation and causes leaf stomata to close and diminish leaf growing to die down parchedness. More modest plates can make harm photosynthesis, which decreases dry matter gathering and grain yield. Due to the weakened compound movement, cell division and development will be diminished without water, bringing about the general decrease of plant organs. Because of the fetus removal of blossoms, the grain yield of vegetables has additionally decreased (Nehl et al., 1997).

Plants generally react to dry season by expanding the measure of saturation in their tissues, expanding their osmotic potential. Insufficiency can likewise cause an increment in receptive oxygen

species in plant tissues. Proline is an amino corrosive whose plenitude expansions without water. Under tension, it can go about as both a saturate and a scrounger of ROS. As a rule, PGPR can work on the exhibition of plants in dry conditions by radiating invade that expands plant osmotic potential.

Another system to further develop dry season obstruction is the blend of gainful chemicals (IAA) and catalysts (ACC deaminase) in plants and the decrease of pressure related chemicals (like ethylene and ABA). Navid et al. revealed that two corn assortments presented to dry season showed diminished harm when vaccinated with two distinct PGPRs, which might be because of chemicals created by microbes and stress-lessening proteins orchestrated by plants and microorganisms during the cooperation-brought about by. Wheat plants immunized with different PGPRs have expanded protection from salt and dry spell treatment identified with the lessening of ABA and ACC levels in plant tissues. In a comparable report, when vaccinated with *Bacillus amyloliquefaciens*, the endogenous ABA level of rice plants diminished and the biomass expanded; the creators speculated that the immunization expanded the salt resistance of the plant through an ABA-free way, which Prevents pressure subordinate ABA amassing and the subsequent development problem (Czaban et al., 2007).

Sarrigetal reported that when *Azospirillum brasilense* colonized sorghum plants, they were presented to osmotic pressure after development and showed decreased harm. Nonetheless, it is hazy whether these perceptions are dry spell explicit reactions or circuitous

impacts of vaccinated plants, showing better root advancement and higher water powered conductivity under pressure. In two sequential investigations of different *Azospirillum* species, vaccinated wheat plants exposed to dry spell have diminished grain misfortune, better dampness status, and higher K. They Ca content, particularly showing the last Participated in adjusting to the effect of plants on ecological pressing factor. Bacterial nitrate reductase is additionally accepted to assume a fundamental part in plant nitrate absorption under dry spell conditions.

As referenced before, dry spell and salt pressure are connected on the grounds that salt is generally the consequence of water system practices to stay away from lack of hydration of plants because of dry season pressure. This worry may turn out to be much more basic in the following not many years, as higher temperatures brought about by an unnatural weather change will prompt higher evapotranspiration and require expanded water system. By 2050, half of developed land might be seriously influenced by salinization. Further developing plant protection from dry spell conditions will decrease the requirement for water system and by implication work with the continuous salinization interaction of farmland.

5.3. Improvement of the plant nutritional status

In the regular habitat, plants bite the dust and deteriorate where they develop, and the ensuing garbage once again introduces the vast majority of the supplements they ingest during development into the dirt. These supplements are eliminated at collect in arable land and

should be continually recharged to stay away from a decrease in efficiency. Among the macronutrients, nitrogen, phosphorus and potassium are the most significant for plant development and are generally once again introduced utilizing engineered manures. Be that as it may, the unequal utilization of compound composts diminishes soil quality, burns-through natural material saves, and expands the danger of disintegration. Soil microscopic organisms can straightforwardly work on the dietary status of plants by expanding supplement bioavailability and by implication by further developing plant root improvement to investigate higher soil regions (Reinhold et al., 1985).

5.4. N₂ Obsession and Assimilation

A few bacterial animal categories are named nitrogen-fixing microbes, which use nitrogenase to fix climatic N₂. Nitrogen-fixing microorganisms can tie to the host to fix N₂ in a free-living structure or as an endosymbiont. The most all-around portrayed cooperation among plants and nitrogen-fixing microbes is rhizobia-vegetable beneficial interaction. A rhizobium is a gathering of different proteobacteria that can colonize plant roots, fix nitrogen in the air, and mostly give it to plants to trade photosynthesis. Albeit this affiliation is primarily seen in beans, a few rhizobia can likewise colonize beans. Gutierrez-Zamora and Martinez-Romero showed how co-developed corn and vegetables share similar rhizobia strain, and vegetables may comprise the wellspring of corn immunization. The connection with rhizobia builds the biomass of the two yields, yet in corn, this outcome might be identified with systems other than nitrogen obsession, like chemical

creation. Vaccinate rice with *Azoarcus* sp. The colonization of wild-type strains and freak strains inadequate in the nitrogenase quality showed further developed development. When spring wheat and corn were vaccinated with two unique rhizobia and developed under various soil nitrogen levels, these two strains could just adequately advance plant development at low and medium soil nitrogen levels. Microscopic organisms make the underlying foundations of immunized plants better create and retain more supplements from the soil (Kawasaki et al., 2016).

As a rule, nitrogen-fixing microbes identified with oat roots ordinarily convey the nitrogenase quality important to fix climatic nitrogen, yet related compounds are not generally blended in plant tissues. What's more, the measure of fixed nitrogen gave to plants is generally irrelevant because of the low substance of nitrogen-fixing microbes or on the grounds that the microscopic organisms utilize fixed nitrogen for their development. Nitrogenase can't work within the sight of O₂, so it very well might be important to configuration free-living nitrogen-fixing microorganisms that can colonize plant tissues. Other potential techniques might be to expand the quantity of fixed microbes through designed plants that can exudate helpful nitrogen-fixing compounds or designed microscopic organisms that can give higher nitrogen levels.

Fox et al. Changed the *Pseudomonas* family. Work on the presentation of wheat and corn by adding quality groups with nitrogenase movement and immobilizing N₂. This is an illustration of the nitrogen obsession technique for vegetables; plants can acquire the nitrogen required for

development from the climate through endosymbionts. For quite a long time, ranchers have profited with the advantageous connection among rhizobia and vegetables. Stretching out this element to vegetables will have definitive advantages for present day agribusiness and give feasible, environmental, and monetary manageability for the wellspring of the main harvest of nitrogen.

5.5. Improvement of Soil Supplements Take-up

In spite of the fact that PGPR is gainful to plant supplement content, it is for the most part muddled whether this improvement is identified with expanded mineral assimilation or regardless of whether it results from further developed root advancement of vaccinated plants because of bacterial chemicals and enzymes (Reinhold et al., 1985).

Because of the mineralization of natural phosphate and the disintegration of inorganic phosphate, different bacterial strains are known to expand the bioavailability of phosphorus in the dirt. A portion of the bacterial mixtures identified with these two cycles are corrosive phosphatase and natural corrosive. PGPR can likewise orchestrate siderophores, which are low-sub-atomic weight compounds with high iron-restricting proclivity, which can be compounded with Fe (primarily Fe³⁺) in the dirt. The iron-siderophore complex is then absorbed by microbes utilizing complex-explicit receptors. This has an assortment of impacts. In the first place, it burns-through the iron inventory in the dirt, along these lines forestalling the development of other possibly pathogenic microorganisms. In addition, in case iron is given to plants, it can straightforwardly further develop plant

development. Moreover, bacterial nitrogenase movement and if quality articulation relies upon iron; accordingly, iron retention in the dirt permits nitrogen-fixing microbes to change over climatic N₂ into a structure that plants can utilize.

PGPR can in a roundabout way further develop plant execution and kill pressure related chemicals delivered by plants in helpless soils. For instance, wheat plants developed under various N, P, and K levels showed expanded grain yield and biomass yield when colonized by the *Pseudomonas* greenery, and bacterial development advancement was adversely connected with the quantity of supplements gave. The creators quality this outcome to the bacterial creation of ACC deaminase, which lessens the degree of ethylene delivered by plants because of low supplement levels, which can hinder root advancement in uninoculated plants.

Generally speaking, plant development advancement is ascribed to a blend of various components. Egamberdiyeva immunized corn seeds with PGPR with nitrogenase and IAA action and planted them on two soil types with various supplement usage. Vaccinated plants for the most part foster higher root and shoot biomass and have higher N, P, and K substance. Plants developed on supplement helpless soils have more huge upgrades. Nonetheless, this examination didn't consider potential associations between the immunized strains and neighborhood microbial populaces that may influence the outcomes.

From 2014 to 2015, half of the 182 million metric tons (Mt) of compost devoured was utilized in beans. Beans finish the greater part of nitrogen

composts and more than 33% of phosphorus and potassium manures. As referenced before, these changes have high monetary and ecological expenses since they cause soil corruption, water contamination, and eutrophication. Albeit the advancement of nitrogen-fixing PGPR is an incomplete errand for grain agribusiness, there is plentiful writing to demonstrate how PGPR can work on the proficiency of harvest retention of supplements. This can occur by expanding the bioavailability of supplements in the dirt or because of better root improvement, prompting better soil investigation.

6. ISSUES AND PERSPECTIVES

In the previous few decades, grain-PGPR associations have been broadly contemplated, and their beneficial outcomes on plant development are as yet being set up. Notwithstanding, the absence of consistency between various investigations is as yet an issue, stressing that speculations can't be made when numerous organic entertainers are included. At the point when the development conditions are ideal, similar bacterial strains may profit one plant and harm another or have no impact on plant execution or even be unsafe, yet become valuable when the development conditions disintegrate. In two examinations on water-inadequate maize and rice, the useful impacts of different bacterial confines on plant development expanded with the seriousness of the pressure. Solano et al. contemplated the communication among PGPR and *Cistus ladanifer*. It is conjectured that a potential clarification for this is that cruel conditions may weaken the development of nearby microbial networks, subsequently decreasing rivalry for

microorganisms that have useful associations with plants. Another conceivable clarification is that when the essential bacterial instrument that advances plant development gives them supplements, the advantages might be restricted in supplement rich soils however might be critical when supplements are restricted.

Noticed outcomes are particularly from research facility and climatic room examinations to more open settings, like nurseries and fields, where microbes typically neglect to further develop plant development. Until this point in time, most examinations on the communication among heartbeats and PGPR have been led in a controlled climate, generally applying just each sort of pressing factor in turn. Albeit this is a fundamental trade off when beginning to contemplate this communication, it normally prompts critical deviations from the real field climate, where plants frequently face seriously changing development conditions and face various pressing factors at the same time. Consequently, the extraordinary reaction of plants is not quite the same as the amount of the responses of plants to singular pressure. Up until this point, scarcely any investigations have contemplated the association among microbes and harvests under numerous pressing factors, however reproducing real field conditions as precisely as conceivable is a vital advance to comprehend and use the job of PGPR in farming. Notwithstanding the more temperamental development climate, another significant variable added to the field tests is the connection with the local microbial local area. Contrasted and lab, climatic chamber, and nursery tests, field-immunized microbes

ordinarily show a lower rhizosphere or root colonization rate. In this manner, the development medium is typically cleaned toward the start of the examination.

One of the speculations that can be drawn from the current writing is that the wellspring of the immunized microscopic organisms is frequently the definitive factor in the collaboration that advances plant development. Microscopic organisms separated from a similar plant animal types utilized in the analysis are bound to assume a gainful part, perhaps in light of the fact that plant-explicit discharges assume a critical part in the beginning phases of the cooperation. Additionally, microscopic organisms disconnected from conditions (particularly temperature) like the conditions and pressing factors forced in plant investigations might be more gainful than microorganisms confined from ideal conditions since transformation permits microorganisms to be more useful than the normal microbiota Intensity. Tragically, because of constant development in a research facility climate, the inoculum utilized in the analysis may turn out to be less powerful. This ought to be considered when arranging the plant explore. One of the issues confronting the commercialization of PGPR in the market is plant immunization conveyance. In the research center, a typical technique is plunge vaccination, in which the foundations of the seedlings are drenched in bacterial culture and afterward relocated into the development substrate. In any case, this technique isn't plausible in field-scale yearly vegetables. Albeit the field use of the bacterial arrangement in the wake of seedling germination isn't so arduous, it

actually requires extensive hardware and specialized information. Presumably the most doable approach to apply PGPR in the field is to utilize pre-vaccinated seeds (which have been utilized for rhizobia-vegetable immunization), permitting ranchers to plant in bunches, accordingly liberating them from the vaccination step. At the point when the seed bacterial therapy is completed preceding germination, the necessary bacterial inoculum power is generally not exactly that of the seedling treatment, yet preferably, the inoculum ought to get by on the seed coat sufficiently long to exist during germination; nonetheless, the microorganisms are drawn out the endurance season of treated seeds is as yet a test. Likewise, irregularities in the exhibition of seed inoculants are frequently seen in various analyses, and further examination is expected to take care of this issue. The utilization of vertical exchange of microbial endosymbionts in seeds may likewise give a potential immunization method that has not been broadly investigated and may furnish ranchers with financial advantages and lighten the vaccination feasibility problem of the seed coat. As of late, concentrates on bacterial strains that spread upward in grain seeds have shown promising plant development advancing impacts, which might be identified with their capacity to disintegrate phosphorus, produce chemicals, siderophores, and ACC deaminase. Utilizing existing collaborations among plants and known seed endophytic microorganisms or separating new strains fit for parasitizing in seeds for vertical transmission through crops, new innovations with huge scope monetary applications may arise.

In the previous few decades, the expansion in efficiency in supplement rich conditions has advanced harvest choice, and little consideration has been paid to the beneficial outcomes of PGPR. In any case, this pattern may prompt the deficiency of plant attributes identified with microbial cooperation. Furthermore, the renewed introduction of qualities identified with these characteristics may upgrade the constructive outcomes of PGPR, particularly in cruel conditions. Along these lines, plant reproducing projects ought to consider choosing plants that communicate well with the rhizosphere microbiota. Furthermore, a more straightforward approach to assuage temperature pressing factor might be to vaccinate plants with microscopic organisms from blistering environment areas, so it is bound to assist their hosts with performing hotter conditions.

Because of the hereditary pliancy of microorganisms contrasted and plants, and considering the effect of environmental change, the cooperation with microorganisms will get more consideration later on. Thusly, PGPR may advance quickly and foster powerful transformation techniques that are helpful to plant.

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