Use of Some Bacteria and Mycorrhizae as Biofertilizers in Vegetable Growing and Beneficial Effects in Salinity and Drought Stress Conditions

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Abstract

Industrialization and rapid population growth, especially after the second half of the twentieth century, have also revealed significant environmental problems in the world. The consistent and alarming increase in the human population has again threatened the world's food security. It is becoming increasingly clear that conventional agricultural practices cannot sustain the production base, a healthy plant-soil system, for too long. There is a growing worldwide demand for compatible environmentally friendly techniques in agriculture, capable of providing adequate nourishment for the increasing human population and of improving the quality and quantity of certain agricultural products. For these reasons, the application of beneficial microorganisms is an important alternative to some of the traditional agricultural techniques which very often severely alter the agroecosystem balance and cause serious damage to health. Beneficial microorganisms can play a key role in this major challenge, as they fulfill important ecosystem functions for plants and soil. Utilization of these microorganisms affects plant's growth and yield in a positive way. Besides, their favorable effects on root growth help plants to deal with both biotic and abiotic stress factors. PGPR and mycorrhizae can influence higher plants response to abiotic stresses such as drought and salinity through different mechanisms.

Keywords: bio-fertilizers, PGPR, mycorrhizae, vegetable, abiotic stress, salinity, drought

1. Introduction

No matter chemical fertilizers or manures, using fertilizers for the purpose of improving the fertility of the soil and the productivity of the crops have caused that the biogeochemical cycles in the nature have been affected negatively [1, 2], and the nutrients (specifically nitrogen (N) and phosphorus (P) were run off, which ultimately caused degradation in the environment [3, 4]. There are several underlying reasons for this situation some of which are the low use-effectiveness of fertilizers and the constant long-term use. Although there are damaging environmental effects, it is expected that the total fertilizer amounts that are used in the whole world will increase in future due to the ever-increasing world population, because there appears a need for producing more food by applying intensive agriculture, which necessitates a great amount of fertilizers [5, 6].

There are two objectives in modern horticulture that contradict with each other: the need to provide food for ever-rising population of the world; and the need for minimizing the damage done to the environment, which can affect horticulture in a negative way [7]. In this respect, horticultural industry and scientists face a major sustainability challenge [8]. In the past 10 years time period, there were some innovations in the field of technology to improve the sustainability of the production systems by reducing the use of chemicals. "Biostimulants" have been proposed as an effective tool in this context. As a result of the efforts made to reduce the harmful effects of fertilizers, plant growth promoting rhizobacteria (PGPR) and/or arbuscular mycorrhizae fungi (AMF) have been proposed as complements for fertilizers. "Plant biostimulants contain substance(s) and/or microorganisms whose function when applied to plants or to rhizosphere is to stimulate natural processes to enhance nutrient uptake, effectiveness, tolerance to abiotic stress, and crop quality, with no direct action on pests."

The rhizosphere is a soil volume under the effect of plant roots. Hiltner [9] defined "rhizosphere" as a maximum microbial activity zone. The microbial population that exists in this medium is different from the population that surrounds it because of the root exudates, which act as nutrition source for microbial growth [10]. The microorganisms may exist in the rhizosphere, rhizoplane, root tissue, and/or in a specialized root structure that is named "nodule." Among the plant, soil, and microorganisms that exist in the soil medium, significant interactions were reported [11]. These significant interactions can be beneficial, neutral, and/or harmful, and may affect growth of plants [12–14]. Usually there are bacteria, algae, fungi, protozoa, and actinomycetes in the microorganisms that colonize in the roots of plants. Evidence has been presented about the enhancement of plant growth and development by applying these microbial populations [15–19]. Bacterial population, i.e., fungi include a significant portion of soil rhizosphere microflora and affect plant growth. The togetherness of fungi and plant roots (mycorrhizae), which is symbiotic life, enhances the root surface area, and this enables the plant to absorb water and nutrients from big soil volume in a more efficient manner. Two mycorrhizae (ecto- and endo-mycorrhizae) types were reported in a few plant species. The mycorrhizae increase the availability of the nutrients and water, and in addition, protect the plant from some abiotic stresses [20, 21].

Agriculture is influenced greatly by the climate change; especially agriculture in tropical areas face increased stress because of natural and anthropogenic factors. In some major crops, increased abiotic and biotic stress is a major cause for productivity stagnation. It has been considered as a big difficulty to develop efficacious, low-cost, and easy-to-apply methods in abiotic stress management. Many studies have been conducted throughout the world for the purpose of developing tactics to deal with abiotic stress. In such studies, developing species that are tolerant to heat and drought, changing crop cultivation times, resource management, etc., were applied [22]. Many newly introduced technologies are cost-effective. Some studies conducted recently have reported that microorganisms could help crops fight against abiotic stress. It has long been recognized that microorganisms have effects on plant growth, nutrient management, and disease control. Some useful microorganisms invade the rhizosphere/endorhizosphere of plants. They enhance plants via some direct-indirect mechanisms [23]. In addition to these, the role of microbes in biotic and abiotic stress management has been focused on more in recent times. Soil supports plant growth through complex and dynamic systems. Plant growth and development are affected by some stresses which are major constraints for sustainable agricultural production in the soil environment. Biotic stresses include plant pathogens and pests (viruses, bacteria, fungi, insects, and nematodes). Abiotic stresses are salinity, drought, flooding, heavy metals, temperature, gases, and deficiency of nutrients or excessive nutrients. Abiotic stresses cause yield reduction, and their intensity changes according to the soil types and plant factors. Imbalance in hormones and nutritive elements, physiological disorders (epinasty, abscission, and senescence), and susceptibleness to diseases are some of the general impacts of these stresses [24–28].

2. Beneficial microorganisms against stress conditions PGPR and mycorrhiza

2.1. Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) are useful bacteria that act on some soil types and facilitate that plants grow and develop in (in)direct ways. In a direct way, fixed nitrogen, phytohormones, iron isolated by bacterial siderophores, i.e., iron-carriers, and phosphate in soluble form are given to plants. In an indirect way, phytopathogens (biocontrol) are avoided resulting in plant growth enhancement. Such functions are performed by PGPR through several enzymes (like bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase) stimulating physiological changes at molecular level. ACC has an important effect on ethylene regulation, which is a plant hormone, resulting in modified plant growth and development. Bacterial strains with ACC deaminase may eliminate negative effects caused by stress and mediated by ethylene.

It was reported that there was ACC deaminase in some Gram-negative microbial bacteria, Gram-positive bacteria, rhizobia, endophytes, and fungi. It was investigated in some species of plant growth enhancing bacteria (*Agrobacterium genomovars* and *Azospirillum lipoferum*, *Alcaligenes* and *Bacillus*, *Burkholderia*, *Enterobacter*, *Methylobacterium fujisawaense*, *Pseudomonas*, *Ralstonia solanacearum*, *Rhizobium*, *Rhodococcus*, and *Sinorhizobium meliloti*, and *Variovorax paradoxus*).

The ACC of the root is metabolized into α-ketobutyrate and ammonia by the ACC deaminase. It also checks the ethylene production. If this process did not occur in this way, the growth of the plant would be inhibited via some mechanisms. If plants are treated with bacteria that have ACC deaminase, it is possible that they have extensive root growth because of less amounts of ethylene. In this way, plants may resist several stress sources. In recent years, using PGPR with ACC deaminase activity, to improve the growth of plants under stress and normal conditions, has been dealt with researchers as an interesting and new field. Also, cultivars' genetic manipulation with genes that express this enzyme has been dealt with recently by several authors. For this reason, focus must be laid on the further parts of this manuscript on late developments in this field of biotechnology.

Data on biosynthetic pathways of ethylene production in plants enabled us to elucidate the mechanisms by which plants regulate the endogenous ethylene level for their normal growth. It has been demonstrated that S-adenosylmethionine or ACC-degrading enzymes decrease ethylene levels in an efficient manner without changing plant physiology. For this purpose, researchers investigated some enzymes that aid to decrease ethylene levels in plants. In this respect, S-adenosylmethionine (SAM) hydrolase and SAM decarboxylase were examined less with regards to ethylene regulation in plants. ACC synthase and oxidase were examined more with several plants.

The ACC deaminase, which is a pyridoxal 5-phosphate (PLP)-dependent polymeric enzyme, was first investigated in a soil bacteria species *Pseudomonas* sp. strain. Bashan et al. [29] described structure for ACC deaminase and provided an understanding about the working of sole pyridoxal-5-phosphate that depends on cyclopropane ring-opening reactions of this enzyme in *Pseudomonas* sp. It was reported in [30] that there was a wide range (>100-fold) in ACC deaminase activity level in various organisms which show high ACC deaminase activity and typically bind to some plants. In this group, there are rhizosphere, phyllosphere organisms, and endophytes, which may behave as a sink-like structure for ACC that appear as a result of stress in plants. In addition, the abovementioned show little preference for one plant over another. However, the organisms that express low deaminase may only bind to some plants. They may also be expressed solely in some tissues; and do not reduce the level of ethylene in plants; but, they prevent a localized increase in the levels of ethylene. Glick reported that there are some rhizobia and ACC deaminases.

Glick et al. [31] investigated the model of PGPR which includes ACC deaminase. They examined how a bacterial ACC deaminase with a low relation to ACC could cope with plant enzymes and ACC oxidase that has high relation with the same substrate resulting in a reduction of endogenous ethylene concentration of a plant. They claimed that biological activity of PGPR was related with ACC deaminase ACC oxidase amounts. In order for PGPR to decrease ethylene levels in plants, the level of the ACC deaminase must be minimum from 100- to 1000-fold bigger than ACC oxidase level. For this to happen, the ACC oxidase expression must not be induced.

Indole-3-acetic acid (IAA) is synthesized and excreted by PGPR. IAA is adsorbed by the surface or roots of the seeds of plants by tryptophan and some molecules in seeds or root exudates. Plants take up some IAAs that are synthesized recently, and IAAs may stimulate the cell proliferation and elongation of plants. In addition, SAM is converted into ACC by enzyme ACC synthetase stimulated by IAA. In the model of Glick et al., an important deal of ACC can be exuded from the roots or seeds of plants. It may also be taken up by soil microbes. It is also possible that it is hydrolyzed by vital microbial enzyme ACC deaminase to produce ammonia and α-ketobutyrate. This process causes that the ACC amount is reduced outside plants. In addition, the balance between internal-external ACC is kept stable via the exudation of more ACC into the rhizosphere. Soil microbial communities with ACC deaminase activity cause that plants biosynthesize more ACC than the plant could need and arouse ACC exudation from plant roots. Meanwhile, they will also provide microorganisms with nitrogen (ACC). As a result, microorganism with ACC deaminase growth is enhanced near roots of the plants. In this way, the ACC level is reduced in plants, and also, the ethylene (stress hormone) biosynthesis is inhibited. In some studies, PGPR inoculation with ACC deaminase was shown to change the endogenous ethylene levels, which ultimately lead to variations in plant growth.

Several chemicals (aminoethoxyvinylglycine (AVG), aminooxyacetic acid (AOA), and 1-methylcyclopropene (1-MCP)) were used to reduce the ethylene level in plants. They were also used to change the sensitivity to ethylene during fruit ripening and flower wilting. In many situations, these chemical substances are not cheap, not easily obtained, and are harmful for the environment. Using PGPR in a natural soil and plant environment is more economical and feasible and is more economical friendly because PGPR includes ACC deaminase activity. In addition, it has also some other advantages like the ACC deaminase trait being more common in some PGPR species that are native to rhizosphere and have a wide variety of survival potential in rhizosphere and rhizoplane. Moreover, PGPR has some other aspects (such as auxins, gibberellins, cytokines, and/or polyamines syntheses contributing directly to plant growth). These features cause that the selection of PGPR with ACC deaminase is more reliable than other alternatives.

2.2. Mycorrhizae

AMF were first described in the last years of nineteenth century. Albert Bernard Frank described the symbiotic associations between the plant roots and the fungi (mycorrhizae). Mycorrhizae means “fungal root.” This association’s basic principle is the nutrients taken up from the soil are exchanged with sugar. Lots of microorganisms form symbiosis with plants ranging on a continuous scale from parasitic to mutualistic. A typical example of these widespread mutualistic symbioses is the arbuscular mycorrhiza formed between AMF and vascular flowering plants [32]. Many scientists and mycologists researched the relations (associations) between mycorrhizae and the plants biology and their inoculation methods. This relation includes the structure of the root and mycorrhizal inoculation. Mycorrhizae are complex symbioses and the fungi produce some structures in the root. Quantification of the structures (hyphae, arbuscules, and vesicles) was standardized by the method suggested by Hungria and Vargas [33]. An arbuscular mycorrhiza has three important elements; the root, the fungal elements between the cells of the root and an extraradical mycelium in soil [34]. The most common type of mycorrhizae is the arbuscular mycorrhiza occurring in about 90% of plant species infected with mycorrhiza. The most common type of mycorrhizae is the arbuscular mycorrhiza occurring in about 90% of plant species infected with mycorrhiza, approximately 83% of dicotyledons, 79% of monocot, and 100% of gymosperms. Most crop plants form mycorrhizae with the exception of the Brassicaceae (e.g., mustard, cabbage, and canola) and Chenopodiaceae (e.g., sugar beets and spinach).

AM fungi consists approximately 160 species belonging to three families. Glomaceae, Gigasporaceae, and Acaulosporaceae. More than 6000 fungal species can form mycorrhizae with about 240,000 plant species. AMF plants own bigger extraradical hyphae formation and soil aggregation. They enhance tilth and excrete hydrophobic protein called “glomalin.” AMF produce more stress-resistant plants during production and for landscape, they reduce the pesticide usage, they increase the more drought and nutrient tolerant plants in landscape, and they potentially higher transplanting success and faster establishment. A symbiotic association formed by fungi with roots, exchanging for functioning as an extended root system, the fungi receives carbonhydrates from the host plant [35].

Arbuscular mycorrhizae fungi (AMF), which are useful organisms, have a significant role in performance and nutrition with plant mineral intake capacity [36]. AMF symbiosis is especially significant in improving the immobile uptake and indissoluble phosphate ions in soil with the interactions with bi/trivalent cations (especially Ca^{2+}, Fe^{3+}, and Al^{3+} [37, 38]. The main function in this mutualism is the capacity of AMF in developing external hyphae networks that may extend the surface area (up to 40 times) and the explorable soil volume for nutrient intake [39] by producing enzymes and/or excreting organic substances [40]. AMF can excrete phosphatases to hydrolyze phosphate from organic P-compounds [41–43], which enhance productivity under harsh conditions (deficiency of phosphorus; [44]). The extraradical hyphae are considered significant in terms of intake of ammonium, immobile micronutrients (Cu and Zn), and some mineral cations coming from the soil (K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}) [45, 46]. It was demonstrated that AMF enhance plant nutrition (biofertilizers), and interferes with the phytohormone balance of the plants, which in turn affects development of plant (bioregulators) and alleviates the influence of the environmental stresses (bioprotectors). This increases the biomass and yield, and causes shifts in some quality parameters [47].

The horticultural products have high phytochemical elements (carotenoids, flavonoids, and polyphenols) and therefore meet the desires of consumers and authors with their health/benefit influences [48]. Furthermore, AMF also bring tolerance to drought [49, 50] and salinity [51, 52], nutrient deficiency, heavy metal contamination [53] and in adverse soil pH [54, 55].

The AMF life cycle begins with asymbiotic stage (germination of the asexual chlamydospores). This depends on several physical factors (temperature and humidity). AMF retract the cytoplasm without the presence of a plant and turn to the dormant phase because they are obligate biotrophs. However, near the roots of the plants, the presymbiotic phase begins with the ramification of the primary germ tube [56]. Root exudates [57] and specific metabolites (strigolactones) may also induce this [58]. When there is a physical contact with the surface of the root, the fungi build up hyphopodia (appressoria) on the surface. On the other hand, a particular mycorrhizae-specific process occurs in epidermal cells underlying hyphopodia in the plant side. They constitute the pre-penetration apparatus, which is a transient intracellular structure used by the fungi to enter the root [59]. Fungal hyphae host the roots of the plant, firstly, between/through cells with linear/simple-coiled hyphae [60], and then build up high-branch hyphal structures that resemble a tree in plant cell apoplast (the arbuscules which gave the name). *Gramineae* members form vesicles rich in lipid as storage organs [61]. Parallel to the colonization of the root, fungi examines the soil around with its hyphae with which they uptake nutrients, interact with other microorganisms, and colonize roots of nearby plants of the same (or different) species. In this way, plants and their AM fungi are interrelated with each other in a network of roots and hyphae [62, 63]. They can exchange nutrients [64] or signals [65] in this way. Eventually, new chlamydospores are created in the extraradicular mycelium. The cycle of life is ended in this way.

3. The most effective environmental stress factors: salinity and drought

3.1. Salinity stress

Under saline conditions, the changes in soil-water potential cause that plant water intake is reduced as well as the nutritional and hormonal imbalance. In these conditions, proline, glycine betaine, trehalose, polyols, and similar organic solutes accumulate in the body of the plant to preserve the plant from the stress-induced effects with osmotic adjustment, with limiting water loss and diluting the toxic ion concentration [66, 68]. Such an accumulation makes it possible for the plant to maintain osmotic potential for improved water intake. For instance, proline accumulation preserves the plant by adjusting osmotic pressure and by stabilizing many functional units (e.g., complex II of the electron transport system, proteins, and enzymes [69, 70]. There are two mechanisms in which high-concentration soluble salts influence microbes: osmotic effect and specific ion effect. Osmotic potential (more negative) is increased by soluble salts and draws water out of the cells, which in turn, may kill microbes and roots via plasmolysis. Because of the low osmotic potential, it becomes more difficult for roots and microbes to eliminate water from the soil [71]. Plants, as well as microbes, can adapt to low osmotic potential through accumulating osmolytes. However, osmolyte synthesis necessitates large amounts of energy, which in turn, results in reduced growth and activity [72, 73]. Certain ions, including Na^+, Cl^-, and HCO^{-3}, are toxic for some plants when they are at high-concentrations [74]. In some previous studies, it was reported that salinity decreases microbial activity and microbial biomass and changes the structure of the microbial community [75–79]. The microbial biomass is decreased by salinity. The reason for this is that osmotic stress causes drying and cell lysis [80–86]. In previous studies, it was also reported that soil respiration was reduced with the increase in the soil EC [87–89]. Gerhardson [90] reported that soil respiration was decreased by more than 50% at EC1:5Z5.0 dS m1. However, according to Glick [91], soil respiration was not correlated at a statistically significant level with EC. However, they also reported that as EC increased, the metabolic quotient (respiration per unit biomass) also increased.

Microorganisms can adapt to/tolerate stress salinity stress by accumulating osmolytes [91–95]. Among the main organic osmolytes, there are proline and glycine betaine; and among the common inorganic solutes, there are potassium cations, which are used as osmolytes accumulated by saline-tolerant microbes [96]. However, high amount of energy is necessary for the synthesis of organic osmolytes [97, 98]. Inorganic salts accumulation (as osmolytes) may be toxic, and for this reason, it is limited to halophytic microbes which developed saline-tolerant enzymes to survive in highly saline medium. Fungi have a tendency for being more sensitive to salt stress than bacteria [99–102]. In this respect, the rate of bacteria/fungi may be increased in saline soils. When compared to nonsaline soils, salinity-tolerance differences among microbes cause those changes that appear in the structure of the community [103, 104].

3.1.1. PGPR help plants tolerate salinity stress

Salt stress enhances endogenous ethylene production in plants and mostly serves as a stress hormone. Probably decreasing the ethylene induced by salinity via any mechanism might reduce the negative effect of salt on the growth of plants. According to recent studies, plants inoculated with PGPR with ACC deaminase could cope with salinity stress with a normal growth pattern. According to Mayak et al. [105], *Achromobacter piechaudii*, which had ACC deaminase activity, increased fresh-dry weight of tomato seedlings at a great deal when grown in with NaCl salt (up to 172 mM). These bacteria decreased the ethylene production in tomato seedlings, and this situation would be stimulated if the seedlings were subjected to increased saline conditions. On the other hand, the sodium level in the plant could not be reduced, and phosphorus and potassium intake was increased. This situation may have enhanced the activation of the events that helped the relief of the side effects of the salt on the growth of the plants. In addition, these bacteria increased the water-use efficiency (WUE) under saline conditions. They also aided in relieving salt suppression of photosynthesis. According to Saravanakumar and Samiyappan [106], *Pseudomonas fluorescens* strain TDK1 that had ACC deaminase activity increased saline resistance of the groundnut plants. The strain also increased the yield when compared with *Pseudomonas* strain inoculation that lacked ACC deaminase activity. Glick et al. [107] verified that ACC deaminase bacteria provided plants with salt tolerance because they lower the salt-induced stress ethylene synthesis and enhance canola growth under saline conditions. We also saw similar results in maize under saline stress as a reaction to the inoculation with ACC deaminase PGPR. The results of research on the physiological effects of some vegetable species related to the benefits of PGPR in salt stress conditions are presented in **Table 1**.

3.1.2. Inducing salinity stress tolerance through inoculation of mycorrhizae

The symbiosis of AM has increased the resilience of the host plants to saline stress, maybe with bigger consistency than to drought stress. Compared to uninoculated controls, growth in saline soils was increased by the inoculation with *Glomus* spp., and with AM plants that had increased phosphate and decreased Na^+ concentrations in shoots [112, 113]. AM colonization in maize enhanced the salt resistance [114], and in mung bean [115] and in clover [116]. The AM influence had a correlation with enhanced osmoregulation/accumulation of proline. The inoculation of AM also enhanced NaCl resistance in tomato with extent of enhancement

References	Used plant growth promoting rhizobacteria (PGPR)	Vegetable species	Stress factor	Result
[108]	N-52/1, N-17/3, FE-43, F-21/3, 637 Ca, MfdCa1	Cucumber	Salinity	FE-43 increased yield 11%.
[109]	*Azotobacter* spp., *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus polymyxa*	Carrot	Salinity	*Azotobacter* spp. significantly increased phenolic content, antioxidant activity, total sugar and soluble solid content.
[110]	*Agrobacterium rubi (strain A16), Burkholderia gladii (strain BA7), Pseudomonas putida (strain BA8), Bacillus subtilus (strain OSU142) Bacillus megatorium (strain M3)*	Mint	Salinity	Root length was observed better in the cuttings were treated with BA7, A16 and M3 compared to the other treatments. Mint cuttings inoculated with M3 had more dry matter content than control and the other treatments.
[111]	N 52/1, N 17/3, Fe 43, F 21/3, 637 Ca	Pepper	Salinity	637 C and N 17/3 in bacteria have demonstrated positive results in practice. Both increased yield, nutrient element uptake and stem diameter.

Table 1. Summary of reported physiologic effects of plant growth promoting rhizobacteria (PGPR) under salinity stress conditions on different vegetables.

regarding the saline sensitivity of the cultivar [117]. AM enhancement of saline resistance was generally related with AM-related increase in P acquisition and plant growth in cucumber [118]. *Gigaspora margarita* colonization enhanced stomatal conductance in sorghum in drought stress in saline soils and also improved the survival dual-stress rates. Evelin et al. [19] investigated whether tomato ("Zhongzha" 105) with *F. mosseae* could increase its salt tolerance. They reported that mycorrhization facilitated salt-related reduction of growth and fruit yield, and also determined that the P and K concentrations were higher and Na concentration was lower in AMF in non-AMF tomato in 0, 50, and 100 mM NaCl. They also claimed that an improvement of the ROS-scavenging enzymes (such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX)) in leaves of salt-affected and control treatment accompanied AMF colonization.

Compared to non-mycorrhizae plants, the bigger antioxidant enzyme activity in plants inoculated with AMF was related with the lower lipid peroxidation accumulation, which indicates lower oxidative harm in the mycorrhized plants. In a similar manner, Habibzadeh et al. [119] reported that enhancement in tolerance to saline stress ("Behta" and "Piazar") of the tomato inoculated with *R. intraradices* was associated with a higher P, K, and Ca intake and with lower Na toxicity. The net photosynthesis enhanced mycorrhization through increasing stomatal conductance and protecting PSII [120]. It was claimed that the increased sink strength of AMF roots was the reason for the mycorrhizae promotion of stomatal conductance [121]. Furthermore, in [122], it was reported that the P, Cu, Fe and Zn accumulation was high in inoculated (*F. mosseae*) than in non-inoculated tomato plants in control and medium salinity groups. However, the Na concentration in the shoot was low in mycorrhized plants, which confirms that the tolerance of the plant to salt stress is enhanced by AMF colonization. Authors [123–125] reported that mycorrhizae pepper ("11B 14" and "California Wonder

300”), inoculated with *Rhizophagus clarum* and *R. intraradices*, had bigger biomass in shoots at different saline concentrations when compared to non-inoculated plants. In non-mycorrhizae plants, the lowest crop performance was reported to be associated with higher Na and lower N, P, K concentrations in leaf tissue and also with high leaf electrolyte leakage, but the effect of the saline stress on pepper shoot biomass varies among different fungi species at a significant level [126]. Cheng et al. [127] reported that inoculation with AMF (*R. intraradices*) might help to beat saline stress in zucchini-squash (*Cucurbita pepo* L. “Tempra”), which is a significant greenhouse vegetable. Enhanced nutrition (higher K and lower Na concentrations in leaf tissue) and the leaf water status might have helped plants to translocate minerals and assimilate to the sink, and alleviate the effects of saline stress on fruit production [128]. It was reported that onion (*Allium cepa* L.) and basil (*Ocimum basilicum* L.) inoculated with AMF could relieve deleterious influences of soil/water saline stress on the yield and growth of crop [129, 130]. About the leafy vegetables, in [131], it was reported that the DAOM 197198 isolate of *R. intraradices* might be accepted as a potential AMF candidate since it stimulated the growth of lettuce under two different saline concentrations. This influence was considered to be linked with higher leaf relative water content and lower ABA in roots, which show that AMF plants are less strained than nonmycorrhizal plants by saline conditions, which enables them to accumulate less ABA. Furthermore, in saline conditions, AM symbiosis improved the LsPIP1 expression, which involved in the transcellular water-flow regulation. A gene expression of this magnitude might contribute to regulate the root-water permeability to tolerate the osmotic stress caused by saline conditions better [132]. Hildebrandt et al. [133] reported in their study that AMF *R. irregularis* alleviated the deleterious influences of saline stress in lettuce (“Romana”) by changing the hormonal profiles (higher strigolactone production) and affecting plant physiology in a positive manner, which allows lettuce to grow better under harsh conditions. Gadkar and Rillig [134] reported that AMF (*G. iranicum* var. tenuihypharum sp. nova) could alleviate the negative influence of irrigation with high saline water on physiological parameters (photosynthesis and stomatal conductance) in lettuce. The results of research on the physiological effects of some vegetable species related to the benefits of mycorrhizae in salt stress conditions are presented in **Table 2**.

3.2. Drought

Climate change is defined as the changes observed over many years in the average state of the climate regardless of its cause. Today’s climate change depends on the greenhouse effect of gases released to the atmosphere due to fossil fuels, improper land use, deforestation, and industrial development, but it is not caused by natural factors, as it has been since the formation of the world. The primary effect of this change, in which the direct human factor plays a role, is the increase in mean surface temperatures, in other words global warming. Modeling efforts to understand global climate change predicts that the average global warming will increase by 1–3.5°C by 2100 and that there will be regional extreme temperatures, floods, and widespread and severe droughts all over the world. Drought is related to the amount of water that can be taken by the roots during the growth period of the plant which is added to the field rather than the total amount of rainfall that occurs throughout the year. Plants that are experiencing water deficiency during the growing period face with significant losses in terms of development and especially yield [143, 144]. Measures should be taken as soon as possible

Reference type	**Used mycorrhizae species**	**Vegetable species**	**Stress factor**	**Result**
[135]	*Glomus clarum*	Pepper	Salinity	Activity of catalase (CAT), glutathione reductase (POD), and ascorbate peroxidase (APX) in leaves of plants treated with mycorrhizae increased. Leaf water potential and osmotic potential has increased. Pepper plants inoculated with mycorrhizal fungi showed the highest chlorophyll content and leaf area in saline conditions. The interaction between mycorrhizal fungi and plants occur higher photosynthesis activities and transpiration rates pursuing with stomatal conductivity.
[136]	*Glomus deserticola*	Spinach	Salinity	*Glomus deserticola* increases K/Na ratio up to 54%.
[137]	*Glomus fasciculatum*	Tomato		MDX levels have increased in plants treated with *G. fasciculatum*.
[138]	*Glomus occultum*	Pepper		Increase in hormone levels of pepper plants with *G. occultum*
[139]	*Glomus fasciculatum*	Cucumber		*G. fasciculatum* caused important changes in the plant enzyme levels.
[140]	*Glomus mosseae*	Pepper		*G. mosseae* significantly increased yield and nutrient element uptake according to control.
[141]	*Glomus mosseae*	Radish		Caused important changes in the plant enzyme levels.
[142]	*Glomus mosseae*	Mint		More ACC deaminase has been detected in plants treated with *G. mosseae*.

Table 2. Summary of reported physiologic effects of mycorrhizae under salinity stress conditions on different vegetables.

to mitigate the effects of agricultural drought, since the available water resources are limited and the occupancy rate of these reserves is predicted to decrease rapidly due to the global warming-related rainfall and especially the decrease in the amount of snowfall that feeds groundwater resources. Although plant varieties belong to the same species, they may differ in their tolerance to drought.

Plants can adapt their growth and development mechanisms in such a way that they are least likely to be affected from environmental changes, and even adapt to environmental conditions when they grow in the same climatic conditions for long periods of time. Drought is one of the abiotic stress conditions which mostly affects the growth and development of plants [145]. Water constitutes 50% of the fresh weight of the trees and 89–90% of the other plants [146]. Plant growth is affected considerably in arid conditions. This effect in growth depends on the length of time the water stress is experienced. In the early stages of arid conditions, the plant slows elongation and triggers root development to reach more water. On the other hand, if arid conditions last long enough to cause damage to the plant, both stem and root growth will stop, leaf area and number of leaves will decrease, and even some leaves turn yellow. The decline in plant growth is due to the division of cells in the shoot and root meristems and the arrest of expansion of the cells. The disruption of cell division or enlargement is directly related to the decrease in the rate of photosynthesis due to water insufficiency [147]. When the plants are exposed to drought stress, the water balance between the tissues is disturbed. In case of stress, cell growth is negatively affected by the loss of turgor, so the cells remain small. The decrease in cell growth also affects the synthesis of the cell wall. While protein and chlorophyll are adversely affected, it is observed that the seeds lose their germination ability [148–150]. Photosynthesis and respiration slow down and stop. Decrease in cell growth causes the leaves to shrink and the production of photosynthesis to decrease further [151]. Water deficiency causes the formation of various reactive oxygen derivatives (ROD) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and superoxide radical (O_2^-) [152]. ROD damages membrane lipids, nucleic acids, proteins, chlorophyll, and macromolecules in the cell. The effect of free oxygen radicals on the cell membrane depends on lipid peroxidation. Lipid peroxidation, which leads to cell membrane destruction, produces malondialdehyde (MDA) as a result of several reaction steps. Drought stress also has an important effect on enzyme activity and enzyme amount in plants. In addition, the amount of abscisic acid is 40 times higher in the leaves, while in other organs including the root, this increase is less. Abscisic acid prevents the transpiration of water by closing the stomata [153].

3.2.1. Inducing drought stress tolerance through inoculation of PGPR

Drought affects almost every climatic region in the world and more than half of it is prone to drought each year. Drought limits the growth and the production of crops as one of the most important stresses. The response to drought by plants is at cellular and molecular level. Drought stimulates the ethylene production in the tissues of plants as it is the case in some other environmental factors and also causes abnormal growth in plants. According to [154], ACC deaminase PGPR *Achromobacter piechaudii* ARV8 increases the fresh-dry weights in tomato and pepper seedlings at a great deal under transient water stress. Also, these bacteria decreased the ethylene production in tomato seedlings under water stress. In water stress, the bacteria had no effects on the water content of plants, and enhanced the recovery of plants if irrigation was started again. It is interesting that when bacteria were given to the tomato plants, the plant growth continued under water stress and also when irrigation was started again. Giri et al. [155] investigated the physiological response of peas (*Pisum sativum* L.) to inoculation with ACC deaminase bacteria *Variovorax paradoxus* 5C-2 in moisture stress and watering conditions. Bacterial effects were more obvious and consistent in controlled soil drying process (moisture stress conditions). In trials that had short time periods, it was seen that ACC deaminase bacteria had positive influences on root-shoot biomass, leaf area, and plant transpiration. In trials that had long time periods, it was seen that the plants that were inoculated with ACC deaminase bacteria produced more seed yields (25–41%), seed numbers, and seed nitrogen accumulations than the plants that were uninoculated. In addition to these, the inoculation caused that the nodulation in pea plants under drought was restored to uninoculated plant levels that were well-watered. In recent years, similar results were reported. According to the recent reports, the inoculation with ACC deaminase bacteria eliminated the influences of water stress on growth, yield, and ripening of *Pisum sativum* L.—although partly—pot and field experiments. The results of the physiological effects of some studies related to the benefits of PGPRs on vegetables in drought stress are given in **Table 3**.

References	Used plant growth promoting rhizobacteria (PGPR)	Vegetable species	Stress factor	Result
[156]	52/1 and E43, 21/3F, 17/3 N, E43 F, 637Ca, MFD Ca1, 52/1, 21/3 + 637Ca, 52/1 Zeatin	Tomato	Drought	21/3F, 21/3 + 637 Ca and 17/3 N bacteria races applications had positive effects on yield and yield components of tomato.
[157]	*Agrobacterium rubi, Pseudomonas putida, Pseudomonas fluorescens, Pantoea agglomerans, Bacillus subtilis, Bacillus megaterium*	Garlic	Drought	*Bacillus subtilis* caused important changes in the plant enzyme levels.
[158]	*Bacillus megaterium* TV-3D, *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK- 92 and *Bacillus megaterium* KBA-10	Broccoli	Drought	PGPR treatments increased seedling length, stem diameter, leaf area, and leaf dry matter at ratios of 7.85%, 42.56%, 18.12% and 41.98%, respectively, compared to the control. Except for Na, the mineral element content was also increased with PGPR treatments.
[159]	*Bacillus megaterium var. phosphaticum*	Tomato	Drought	Plant growth, total and marketable yield increased by *Bacillus megaterium var. phosphaticum*.

Table 3. Summary of reported physiologic effects of plant growth promoting rhizobacteria (PGPR) under drought stress conditions on different vegetables.

3.2.2. Drought stress tolerance through mycorrhizae

Arbuscular mycorrhizae (AM) symbiosis is associated with enhancing the resistance to water and drought stress despite the change of plant physiology and the expression of plant genes [120, 160]. It was reported in previous studies that AM-related increase in drought tolerance involved increased dehydration and dehydration tolerance [161]. AM fungi inoculation was able to reduce the leaf content of malondialdehyde and soluble protein and improve the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which resulted in enhanced osmotic adjustment and drought tolerance of mycorrhizae citrus-grafting seedlings [162]. Inoculation of *Glomus versiforme* in citrus plants enhanced the osmotic adjustment of the plant in drought stress via improved levels of non-structural carbohydrates, $K(^{+})$, $Ca(^{+})$, and $Mg(^{2+})$, which resulted in improvement of drought tolerance [163].

It was reported that the role of abscisic acid (ABA) was behind the AM-related stress response in plants [164]. When exogenous ABA was added, the ABA content was improved in shoots of non-AM plants, concomitant with the expression of the stress marker genes Lsp5cs and Ls1ea and the gene Lsnced. However, when exogenous ABA was added, the ABA content in AM shoots decreased, and this addition did not cause more improvement of the expression. Co-inoculation of lettuce with PGPR *Pseudomonas mendocina* and *G. intraradices* or *G. mosseae* improved an antioxidative catalase in serious drought, which shows that they might be used in inoculants to relieve the oxidative harm [165]. A 14-3-3 protein encoding gene from *Glomus intraradices* growing in vitro and subjected to drought stress was identified [166]. The role of these proteins regulating the signaling pathways and effector proteins was claimed to impart the protection to the host plants against drought stress. Glutathione and ascorbate have a significant effect in conferring the protection and maintaining metabolic function of plants in water deficit conditions.

AMF are known to have an efficacious and sustainable mechanism. With this mechanism, tolerance to drought is enhanced in vegetables [167, 168]. AMF cause changes in the roots of plants, especially in length, density, diameter, and number of lateral roots [169]. Improved root structure in mycorrhizae plants allows the extraradical hyphae to extend beyond depletion zones of plant rhizosphere, which makes the water and low-mobile nutrient intake (P, Zn, and Cu) more efficiently under water stress [170].

The AM symbiosis effectiveness in improving drought tolerance was also investigated in vegetables. Open-field tomato (*Solanum lycopersicum* L.) inoculated with AMF (*R. intraradices*) influenced the agronomical and physiological responses of exposure in different drought intensities [171]. Compared to non-inoculated ones, the fruit yield of inoculated plants in severe-moderate-mild drought stresses was high at a statistically significant level by 25, 23, and 16%, respectively. It was reported in this study that high crop performance in inoculated plants was associated with better nutritional status (higher N and P) in connection with the maintenance of leaf water status. Ikiz et al. [172] confirmed this effect on tomato. They showed that the colonization of processing tomato "Regal 87-5" plants by *F. mosseae* and *G. versiforme* might increase marketable yield by 20% and 32%, respectively, when compared with those of non-inoculated plants under mild-heavy drought stress. Greenhouse melon (*Cucumis melo* L. "Zhongmi 3") plants (inoculated with three *Glomus* species: *G. versiforme* and *R. intraradices* and, especially, *F. mosseae*) showed higher tolerance to drought stress than non-inoculated plants. This situation was determined in plant heights, root lengths, biomass production, and net photosynthetic rates [173]. They claimed that the increase in drought tolerance and better crop performance might be associated with the antioxidant enzyme production (SOD, POD, and CAT) and the soluble sugar accumulation by AM symbiosis. Lucy et al. [174] examined the mechanisms which affected the relief of drought by a mixture of *Glomus* spp. from Mexico ZAC-19 (*G. albidium*, *G. claroides*, and *G. diaphanum*) in Chile ancho pepper (*C. annuum* L. San Luis). They reported that ZAC-19 had the potential to be incorporated into Chile pepper transplant systems to relieve the harmful effect of drought in open-field production in Mexico, which was shown by high root-to-shoot rate and leaf water potential. In a similar manner, in [175] it was reported that drought enhanced bigger extraradical hyphae development of *G. deserticola* in bell pepper, and as a result, a high water intake, when compared to non-mycorrhizae plants. It was also reported that AMF symbiosis enhanced lettuce (*Lactuca sativa* L. "Romana") tolerance to drought and recovery. This enhancement was achieved via the modification of the plant physiology and the expression of plants genes [176, 177]. Lettuce, which was inoculated with the AMF *R. intraradices*, gave high root hydraulic conductivity and low transpiration in drought, when it was compared with non-inoculated plants. Authors [178, 179] also emphasized that the plants inoculated with AMF could regulate their abscisic acid (ABA) concentrations in a better and quicker manner than non-inoculated plants, which allows a better balance between leaf transpiration-root water movement in drought stress and recovery [180, 181]. It was reported that inoculation with AMF enhanced WUE in watermelon [182], which shows that AMF improved water intake and resulted in the host plant making

Reference type	Used mycorrhizae species	Vegetable species	Stress factor	Result
[185]	*Glomus mosseae*	Muskmelon	Drought	K/Na ratio has increased in several plant tissues.
[186]	*Glomus mosseae*	Watermelon		Water use efficiency, Leaf water content and leaf osmotic potential has increased.
[187, 188]	*Glomus mosseae*	Lettuce		Endogenous auxin and cytokinin levels are increased in the presence of *G. mosseae*.
[189]	*Glomus occultum*	Cabbage		Yield and quality increased with mycorrhizae.
[190]	*Glomus fasciculatum*	Lettuce		L-arabinose (L Ara), ribose (Rib); D-xylose (D Xyl), L-xylose (L Xyl), adonitol (Ado), beta-methyl-D-xyloside (Mdx) levels increased.
[191]	*Glomus mosseae*	Aubergine		Water use efficiency, Leaf water content and leaf osmotic potential has increased.
[192]	*Glomus caledonium*	Pepper		Activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) in leaves of plants treated with *Glomus* increased.
[193]	*Glomus mosseae*	Melon		Water-use efficiency, leaf water content, and leaf osmotic potential has increased.

Table 4. Summary of reported physiologic effects of mycorrhizae under drought stress conditions on different vegetables.

use of water in a more efficacious manner [183]. This was associated with the mechanisms that could increase transpiration and stomatal conductance [184], and also improve the availability of the nutrients [183]. The results of the physiological effects of some studies related to the benefits of mycorrhizae on vegetables in drought stress are given in **Table 4**.

4. Conclusion

Today, the utilization of natural resources in agriculture comes to the forefront because of improving environmental awareness. The evaluation of the use of natural resources, such as mycorrhiza and a cleaner environment, is important both for economic reasons. Resources are often used as a source of plant nutrition in hydroponics. Given the chemical, the use of mycorrhiza in agriculture is very important in soil. Particularly with the use of mycorrhiza, the use of chemical fertilizers especially consisting phosphorus, can be reduced. As a conclusion, mycorrhizae are important for the growth of agricultural crops as well as healthy ecosystem functions. Many benefits of mycorrhizal symbiosis can be enhanced by changing agricultural practices which may decrease colonization and mycorrhizal abundance [194].

Hydraheaded stress caused by biotic and abiotic reasons is threatening modern agriculture. Several stress types explained in this chapter emphasize ethylene biosynthesis, which prevents plant growth by some tools at molecular level. In this chapter, for the purpose of regulating the plant ethylene, application of PGPR with ACC deaminase is crucial. Several roles of PGPR in saline conditions, in drought, waterlogging, biocontrol, temperature and nutritional stresses and in cut-flower industry and nodulation in legumes were not investigated in detail by researchers. In commercial terms, applying PGPR with ACC deaminase in agriculture may be useful. It may also be an important progress to obtain sustainable crop production and conservation. Because of several drawbacks, genetic modification of plant species is not probable (for example, proprietary rights, trade agreements among countries for genetically modified (GM) crops, and due to the limitations in DNA recombinant technology in some areas in the world). Because of all these reasons, using PGPR with ACC deaminase activity and similar innovations may be a cost-effective and environment-friendly way for sustainable agriculture.

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