
Microbes for Plant Stress Management

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Foreword



Dr. S. Ayyappan
President

As the world marches towards the year 2050 with the predicted population of 9.7 billion, the daunting task faced by the agricultural scientists is to find out methods to feed the growing population with shrinking natural resources. It is also predicted that the demand for food has to be raised by about 60% compared to the current food production. This challenge gets more complicated with the current threat of global climate change resulting in increased temperature, drought, salinity, etc. These abiotic stresses will vigorously affect the agricultural productivity. It has already been observed that yield of crops is reduced due to various stresses. Abiotic stresses predominantly affect the genetic potential of food crops to the tune of nearly 69%. Drought is the most serious environmental factor limiting the production of agricultural crops with serious economical and social impacts. Salinity is another severe environmental stress decreasing crop productivity, mainly in irrigated land, worldwide. Hence there is an urgent need to address the abiotic stresses in order to achieve the goal of increased food production. Certain agronomic and breeding strategies are recommended for mitigating abiotic stresses. Microorganisms could play an important role in adaptation strategies and increase tolerance to abiotic stresses in agricultural crops. The mechanisms adopted by microorganisms to mitigate abiotic stresses in crop plants are many. The information available on the utilization of microbes for plant stress management is limited.

Viewing from the above stated perspective, the book on “Microbes for Plant Stress Management” by Dr. D.J. Bagyaraj and Dr. Jamaluddin is very timely. The book will be a valuable companion to students, teachers, researchers, administrators and policy makers who foresee the need for managing stresses affecting crop productivity using microbes for feeding mankind in the years to come.

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Preface

The world population is predicted to reach about 9.7 billion by the end of the year 2050 and to feed this population the current food production levels have to be raised by nearly 60% with limited land resource. This challenge gets compounded by the threat of global climate change leading to erratic rainfall, drought, salinity, increased temperature, etc. These abiotic stress consequences will seriously threaten the sustainable agricultural production. Hence there is a need to address this issue by all available means in order to achieve the goal of enhanced food production. Certain agronomic strategies like change in sowing dates, alley cropping, zero tillage, etc. have been proposed to mitigate stress. Breeding strategies recommend mitigating abiotic stresses by choice of resistant cultivars, use of hyper-accumulator plants, etc. A lesser explored potential option for abiotic stress alleviation is in the utilization of stress tolerant microbial resources, which have the ability to promote and sustain crop growth during adverse environmental conditions. This approach has gained popularity in the recent years and seems to be a potential option for the future. Thus the present book brings out the role of different groups of microorganisms like plant growth promoting rhizomicroorganisms (PGPR), arbuscular mycorrhizal fungi (AMF), endophytes, etc. in alleviating abiotic stress in crop plants.

The book contains 14 chapters written by distinguished scientists of the country having expertise in dealing with microorganisms and exploiting them for the benefits of mankind. Chapters 1 and 2 deal with exploring microbes from extreme environments and rhizomicrobiome respectively, for use in sustainable agriculture. Chapter 3 describes the recent developments in utilizing microorganisms for abiotic stress management in crop plants. Chapter 4 helps to understand the innate stress management mechanisms in plants. Chapter 5 deals with nanoparticles synthesized by microorganisms for use in agricultural ecosystem. Chapter 6 covers the role of plant growth promoting rhizomicroorganisms in supporting the growth of plants under stressed environment. Chapters 7 and 8 discuss the role of endophytic microorganisms in alleviation of abiotic stress in crop plants. Chapters 9 to 12 cover the role of arbuscular mycorrhizal fungi in alleviating drought and salt tolerance in different crops important in agriculture and horticulture. Chapter 13 is devoted to

bioconversion of municipal solid wastes and its use in soil fertility. Chapter 14 provides an overview of microbial inoculants for quality seedling production in forestry. Thus the book is a comprehensive and detailed analysis of the subject.

We profusely thank all the authors for their keen interest, kind efforts and rich contributions in making this book highly informative and productive. Special thanks are due to Mr. R. Ashwin for his help in bringing out this book. We are also thankful to New India Publishing Agency, New Delhi for taking keen interest in bringing out this book in time.

D. J. Bagyaraj
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Contribution of AMF in the Remediation of Drought Stress in Soybean Plants

Abhishek Bharti, Shivani Garg, Anil Prakash and Mahaveer P. Sharma

Abstract

Amongst various oilseed crops grown across the world, soybean (*Glycine max* L. Merrill) is globally important as a high source of protein and oil for human consumption and is also being used as potential feed for animals. Soybean is grown under rain-fed conditions in the tropics and subtropics in the marginal lands with less amount of external application of chemical fertilizers. The frequent climate variations have created a number of biotic and abiotic stresses, drought being a major abiotic stress which adversely affects the soybean productivity. Thus, to enhance the productivity of soybean, besides managing the nutrients, stress management is of utmost importance. There is a great opportunity of application of microbes especially arbuscular mycorrhizal fungi (AMF) where its application alone or in combination with plant growth promoting rhizobacteria (PGPR) can help in nutrient mobilization and remediation of plant stresses. AMF are a ubiquitous group of soil fungi known to colonize roots of plants belonging to more than ninety per cent of plant families. In this chapter, detailed account of work done on the mycorrhizal symbiosis and its role in alleviation of drought stress has been presented. We have also described mechanisms underlying drought tolerance in AM-colonized soybean plants. The information on understanding interactions between AMF and PGPR and agronomic practices is also provided which would help in the abiotic stress tolerance of soybean plants. In addition, a brief account on the production methods of AMF and availability of AMF inocula is also provided.

Keywords: Commercial AM inocula, Drought, Mycorrhizal fungi, Soybean

1. Introduction

Soybean [*Glycine max* (L.) Merrill] containing about 40-42% protein and 18-22% oil is emerging as one of the fast growing oilseed crop in the world. In India, Malwa plateau of Central India is the hub for soybean cultivation, the total production during 2014 was about 11.64 mt from 10.02 m hectare (Anonymous 2014-15). Although, the spread of soybean in different parts of the country resulted into parallel growth of oil industry but for the past few years soybean is facing climatic challenges where the soybean yields are declining. When compared to other countries the productivity of soybean per unit area is very low in India and productivity stagnated due to recurrence of drought, the low nutrient use efficiency of crop, nutrient deficiency in soil and other biotic and abiotic stresses. According to FAO's report, 2013; drought has become more frequent and intense worldwide and is a major factor causing alterations in plant morphology, physiology, nutrient uptake and metabolism, ultimately affecting the plant's growth and development adversely (Evelin *et al.* 2009). Various studies have shown that drought stress leads to decrease in soybean yield up to 50% (Sadeghipour and Abbasi, 2012).

Numerous mitigation strategies can be used to cope with such impacts. However, most of them being based are either a long-term in nature like breeding drought tolerance varieties or based on use of chemical fertilizers which are currently becoming inaccessible and costly to farmers. Hence there is a need to develop eco-friendly and low cost biological methods for the management of abiotic stress. Microorganisms such as AMF could be successfully exploited for the purpose, as they possess unique properties such as tolerance to extremities, ubiquity, genetic diversity and interactions with soil and crop plants. Besides influencing the physico-chemical properties of rhizospheric soil through production of exopolysaccharides and formation of biofilm, microorganisms can also influence higher plants' response to abiotic stresses like drought through different mechanisms like induction of osmo-protectants, heat shock proteins etc. in plant cells. Use of these microorganisms can help crop plants to cope with drought stress. They also provide excellent models for understanding the stress tolerance, adaptation and response mechanisms that can be subsequently engineered into crop plants to cope with stresses (Grover *et al.* 2010).

Among all, AMF are cosmopolitan in all soil types and form symbiotic association with the roots of over 90 % terrestrial plant species. They have coevolved with plants over 400 million years ago. They improve adaptation capability of plants to different types of stress like drought. The soil surrounding the plant roots, i.e., rhizosphere, is an ecological niche which is integral to many biochemical reactions and is deeply influenced by the root exudates. Here, microorganisms

have multiple roles such as plant growth promotion, degradation of natural or synthetic compounds. Some of them can act as pathogens as well. PGPR are also known to elicit systemic tolerance to abiotic stresses in plants, apart from the plants' own survival mechanisms such as drought avoidance (through reduced transpiration losses following stomatal closure or water storage in plant tissues) and drought tolerance (through accumulation and translocation of assimilates, osmotic adjustments or maintenance of cell wall elasticity). The plants' adaptations to drought stress can cause changes in belowground C input through higher root production and turnover which in turn influences the functional structure and activity of the microbial community in the rhizosphere (Grayston *et al.* 1998). Several ecophysiological studies have demonstrated that AMF symbiosis is a key component in helping plants to cope with water stress, as demonstrated in a number of host plants. Mycorrhizal colonization improves drought resistance of plants as a consequence of enhancing nutritional status, especially P and water status which in turn enhances plant growth and productivity. Further, plants develop their own survival strategies as well to increase tolerance against drought (Bohnert *et al.* 2006). In this chapter, we describe how AMF contribute in combating drought stress, particularly in soybean plant. Mycorrhizal symbiosis can potentially improve water uptake by plants, mainly through improved nutrient uptake which can be attributed to the enhanced absorbing surface area provided by AMF hyphae in the soil together with the fungal ability to take up water from soil having low water potential. Bohnert *et al.* 2005 have studied signaling interactions in the AMF-soybean symbiosis that is expected to regulate the response of mycorrhizal soybean to drought stress.

2. Role of AMF in enhanced drought stress alleviation in soybean

Drought stress affects many physiological plant processes which in turn induce premature senescence in root nodules of legumes including soybean, thereby decreasing their ability to fix atmospheric nitrogen. Various symptoms of drought stress include reduction in leaf size, extension in stem length and root proliferation. These cause disturbance in plant water relations leading to reduced water-use efficiency (WUE). Symbiosis between AMF and most plants helps nutrient and mineral uptake, and increase plant stress tolerance. Studies have established that association of roots of numerous plant species with AMF increased uptake of various minerals from the soil, including water, and also help host plant's ability to grow under conditions of drought stress (Barea *et al.* 2005). However, in the last two decades, comparatively fewer researchers have taken up studies on symbiosis between AMF and soybean (Table1).

Table 1: Influence of AMF on drought tolerance of soybean (past two decades)

Inoculation	Condition/Stress	Reference
AMF- <i>Glomus mosseae</i> , <i>Glomus etunicatum</i> with and without <i>B. japonicum</i>	Different moisture stress /field capacity levels in pots	Aliasghar zad <i>et al.</i> 2006
<i>Septoglomus constrictum</i> , <i>Glomus</i> sp., and <i>Glomus aggregatum</i> & mixture	Water Stress /Drought stress	Grumberg <i>et al.</i> 2015
<i>Glomus intraradices</i>	*Environmental chamber	Porcel <i>et al.</i> 2003
<i>Glomus mosseae</i> , <i>Glomus intraradices</i>	*Environmental chamber	Ruiz-Lozano <i>et al.</i> 2001
<i>G. mosseae</i> , <i>G. intraradices</i> and <i>Piriformospora indica</i>	Water stress maintained by stopped water spraying /Drought stress	Rathod <i>et al.</i> 2011
<i>Glomus intraradices</i>	Water stress maintained by stopped water spraying /Drought stress	Liu <i>et al.</i> 2015
<i>Glomus intraradices</i>	*Environmental chamber	Porcel R & Ruiz-Lozano JM, 2004
<i>G. deserticola</i> , <i>G. etunicatum</i> , <i>G. intraradices</i> , <i>G. fasciculatum</i> , <i>G. mosseae</i> , <i>G. caledonium</i> , <i>G. occultum</i>	**Environmental chamber	Ruiz-Lozano <i>et al.</i> 1995
<i>Glomus intraradices</i>	Leaf osmotic potential variation	Auge <i>et al.</i> 2001

*Environmental Chamber conditions: 70–80% RH, day/night temperatures of 25/15°C, and a photoperiod of 16 h at a photosynthetic photon flux density (PPFD) of 460–500 $\mu\text{mol m}^{-2}\text{s}^{-1}$

** Environmental Chamber conditions: 70–80% RH, day/night temperatures of 25/15°C, and a photoperiod of 14 h. The photosynthetic photon flux density was 500 $\mu\text{mol m}^{-2}\text{s}^{-1}$

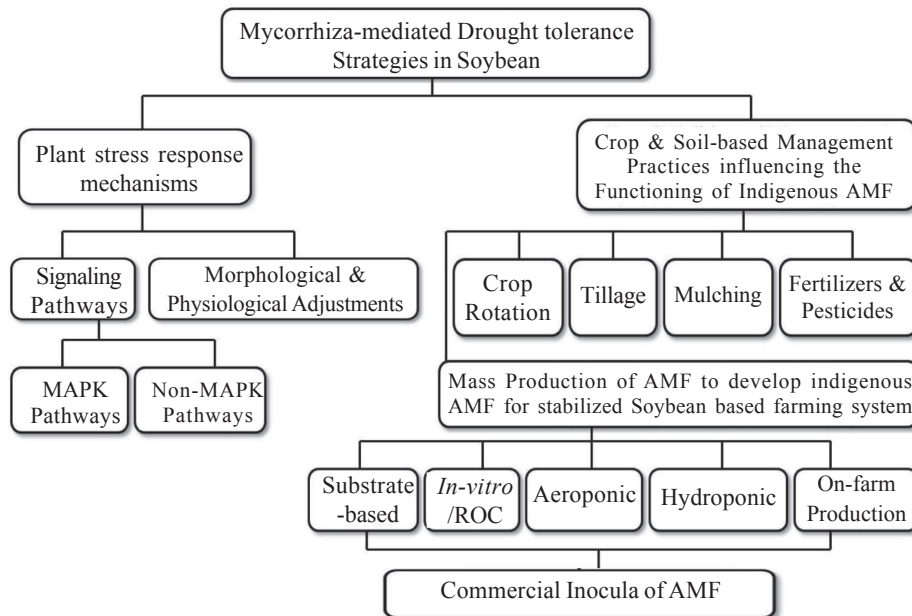


Fig. 1: Mycorrhiza-mediated approaches in providing drought tolerance to Soybean Plants

In soil, water scarcity is tightly linked to low nutrient availability and so various hypotheses have been formulated to explain the underlying plant nutrition mechanisms. Improved nutrient uptake by AMF is a fundamental mechanism that can alleviate the adverse effects of water stress on plant growth (Rapparini and Penuelas, 2014). One of the most common explanations for the improved nutrient status in mycorrhizal plants is the enhanced surface area for absorption that is provided by AMF hyphae (Ruiz-Lozano *et al.* 2003).

3. Mechanisms of drought tolerance

Plants usually interact with soil microorganisms that make it more efficient in coping with environmental stress such as drought. (Azcon *et al.* 2013). The AMF mediated response in soil causes many physiological and biochemical changes in water availability owing to a number of mechanisms that enable drought resistance in plants. Communication between AMF and host plant starts when a spore comes into contact with a host plant root, since the cell walls are the outermost boundary of the root; they are the first interface for interactions with soil-borne microbes. Based on this notion, it has earlier been postulated that interactions of the cell walls between plants and microbes may play an important role in symbiotic interactions. This relation is important for both, plants and AMF. After infection, hyphae penetrate deep into the parenchyma cortex. Here, fungal development culminates in the differentiation of intracellular haustoria, known as arbuscules. These fungal structures, which establish a

large surface of contact with the plant protoplast, are attributed to a key role in reciprocal nutrient exchange between the plant cells and the AMF symbionts. Outer layer of plant root's cell wall participates in many interactions with the soil environment. Evidences suggest that plant itself plays a central role in softening and remodeling of the cell wall during symbioses.

In higher plants, drought stimuli are presumably perceived by osmosensors (that are yet to be identified) and then transduced down the signaling pathways, which activate downstream drought responsive genes to display tolerance effects. The tolerance involves not only the activities of protein receptors, kinases, transcription factors, and effectors but also the production of metabolites as messengers for transducing the signals. Drought tolerance is of multigenic nature, involving complex molecular mechanisms and genetic networks. The signaling pathway of drought stress is similar to those of osmotic stresses that have been reviewed in detail by Ahuja *et al.* (2010).

3.1. MAPK pathway

Mitogen activated protein kinase (MAPK) cascades play important roles in the stress response in both plants and microorganisms. The symbiosis established between AMF and plants can enhance plant drought tolerance, which might be closely related to the fungal MAPK response and the molecular dialogue between fungal and soybean MAPK cascades. Many studies support the role of protein kinases in stress signaling (Bartels *et al.* 2010). In plants, the drought responsive signal transduction of the MAPK family (MAPK, MAPKK, MEKK, MAPKKK, MKK) as well as the MAPK phosphatases (MKP) family have been relatively well-studied in *A. thaliana* and rice but remained under-explored in soybean, although a PA-responsive MAPK has been identified in soybean (Lee S *et al.* 2001). In soybean, two-component histidine kinases (GmHK07, GmHK08, GmHK09, GmHK14, GmHK15, GmHK16 and GmHK17) and receptor-like protein kinases (GmCLV1A, GmCLV1B, GmRLK1, GmRLK2, GmRLK3 and GmRLK4) have been identified as candidates of osmosensors. However, direct evidence for their functions to perceive stress signals in soybean is still missing. Drought stress upregulates the level of MAPK transcripts in mycorrhiza-colonized soybean roots. Hence, there might exist a molecular dialogue between the two symbionts to regulate the mycorrhizal soybean drought-stress response. Meanwhile, the changes in hydrogen peroxide, soluble sugar, and proline levels in mycorrhizal soybean as well as in the accelerated exchange of carbon and nitrogen in the symbionts were contributable to drought adaptation of the host plants. Thus, it can be preliminarily inferred that the interactions of MAPK signals on both sides, symbiotic fungus and plant, might regulate the response of symbiosis and, thus, improve the resistance of mycorrhizal soybean

to drought stress. (Zhilei *et al.* 2014). Extensive study has demonstrated AM-mediated plants are resistant to drought conditions, but the underlying mechanisms have not yet been clearly elucidated.

3.2. Non-MAPK signaling pathways

On the other hand, some non-MAPK type protein kinases found in soybean may be related to drought responses. ABA-mediated signaling pathway: Abscisic acid (ABA) helps plants' response to abiotic stresses by regulating their physiology (Zhang *et al.* 2006). Its biosynthesis, accumulation, and catabolism are all crucial for the transduction of ABA-mediated signals. It is synthesized in various cell types including root cells, parenchyma cells, and mesophyll cells. Under drought stress, ABA is transported to guard cells to control stomatal aperture (Wilkinson S *et al.* 2010). ABA reaching the target tissues and cells will be recognized and the signals will be transduced down the ABA signalosome (Umezawa *et al.* 2010), including ABA receptors (PYR/PYL/RCAR), negative regulators (e.g. group A protein phosphatases 2C) and positive regulators (e.g. SnRK-type kinases). Components of this system have been discovered in soybean. For example, GsAPK is a SnRK-type kinase from wild soybean that is up-regulated by drought stress in both leaves and roots, but down-regulated by ABA treatment in roots (Yang *et al.* 2012). Accumulation of ABA in response to drought is associated with changes in Ca^{2+} (second messenger) and reactive oxygen species (ROS) levels. Drought-induced changes in cytosolic Ca^{2+} level activate the signaling pathways for downstream stress responses (Xiong *et al.* 2002). Various types of Ca^{2+} -binding proteins: CaMs (calmodulins), CMLs (CaM-like proteins), CDPKs (Ca^{2+} -dependent protein kinases), and CBLs (calcineurin B-like proteins) (Falco *et al.* 2010) act as Ca^{2+} sensors.

Expression of the soybean CaM (GmCaM4) in transgenic *A. thaliana* activated a R2R3 type MYB transcription factor which in turn up-regulated several drought-responsive genes, including *P5CS* (encoding a proline anabolic enzyme) (Yoo *et al.* 2005). While the application of Ca^{2+} affects the nodulation of soybean (Bell *et al.* 1989), the gene encoding a soybean CaM binding protein was found to be differentially expressed in soybean nodules under drought stress (Clement *et al.* 2008). In isolated soybean symbiosome membrane, a CDPK was demonstrated to phosphorylate an aquaporin called nodulin 26 during drought conditions and hence enhance the water permeability of the membrane. (Guenther *et al.* 2003). Besides Ca^{2+} , phosphatidic acid (PA) and the intermediates of inositol metabolism are also second messengers for signal transduction (Xue *et al.* 2009). However, there are only very limited evidence supporting the involvement of phospholipid signaling in drought stress response of soybean.

Cellular ROS gets accumulated during drought stress that triggers the generation of hydrogen peroxide, a signaling molecule that will activate ROS scavenging mechanisms (Cruz *et al.* 2008). In soybean, exogenous application of hydrogen sulphide alleviates symptoms of drought stress, probably *via* triggering an antioxidant signaling mechanism (Zhang *et al.* 2010). The ubiquitin-mediated protein degradation pathway: this is also an integral part of the signal transduction network (Zhou *et al.* 2010). This pathway directs the degradation of target proteins by the 26S proteasome and is responsive to drought stress. Two ubiquitin genes and one gene encoding ubiquitin conjugating enzyme were identified as differentially expressed genes in nodulated soybean under drought stress (Clement *et al.* 2008). Over expression of the ubiquitin ligase gene *GmUBC2* enhances drought tolerance in *A. thaliana*, *via* up-regulating the expression of genes encoding ion transporters (AtNHX1 and AtCLCa), a proline biosynthetic enzyme (AtP5CS), and a copper chaperone (AtCCS) (Zhou *et al.* 2010).

3.3. Morphological and physiological adjustments of soybean under drought stress

Various morphological traits indicate drought stress response in soybean (Liu *et al.* 2005). For instance, root distribution, which is measured in terms of horizontal and vertical root length or dry matter in soil of different depths (Benjamin and Nielsen, 2006). Liu *et al.*, 2005 have also reported a positive correlation between drought tolerance and dry root weight/ plant weight; total root length/ plant weight, and root volume/ plant weight. Further, the root to shoot ratio increases under water deficit conditions (Wu and Cosgrove, 2000), indicating a higher sensitivity of shoot than roots towards drought conditions. This happens due to differential changes in cell wall composition involving thickening of shoot cell wall and expansion of root cell wall by catalytic enzymes and stiffening agents. However, there are only limited reports on related studies in soybean. The study on GmRD22 from soybean suggested a relationship between osmotic stress and cell wall metabolism. GmRD22 is a BURP-domain containing protein localized in the apoplast, which may play a role in stress tolerance by regulating lignin content of cell wall under stress, presumably through interacting with peroxidases of the cell wall (Wang *et al.* 2012). Changes in leaf morphology may also play a role in drought tolerance. Some cultivars take advantage from the maintenance of leaf area which provides a possible benefit for the growth of soybean plant after the stress is relieved (Manavalan *et al.* 2009). Under stress, drought tolerant soybean cultivars exhibited a larger leaf area when compared with less tolerant cultivars; this can be attributed to a reduction in stomatal conductance and photosynthetic rate in the tolerant cultivar (Stolf *et al.* 2010).

3.4 Physiological adjustments

Plants undergo physiological adjustments at various levels to cope up with drought stress. For instance, it is important for the soybean leaves to adjust stomatal conductance to prevent excessive water loss. Stolf *et al.* 2010, reported a higher degree of reduction in stomatal conductance as compared to drought sensitive cultivar BR 16, 30 days after water stress. Maintenance of cell turgidity is another crucial adjustment. To maintain cell turgidity under stress, osmotic adjustment is a common mechanism which involves active accumulation of solutes in cells (Manavalan *et al.* 2009). Drought tolerant soybean cultivar PI 416937 has been found to maintain a lower solute and higher water potential as compared to sensitive cultivar Forrest, which in turn resulted in a higher seed weight and yield than Forrest under drought (Sloane *et al.* 1990). In soybean, drought stress up-regulates the expression of the *P5CS* gene which encodes the enzyme Δ^1 -pyrroline-5-carboxylate synthase, a key enzyme in proline biosynthesis (Porcel R *et al.* 2004). Knocking down this gene hampered survival under drought stress (Ronde *et al.* 2000). However, the involvement of proline accumulation in drought stress adjustment in soybean awaits further confirmation. Cellular biochemical adjustments under drought stress involve scavenging of ROS. Under normal situation, ROS including singlet oxygen, superoxide radical, hydrogen peroxide, and hydroxyl radical are continuously synthesized and eliminated in plant cells as “by-products” of photosynthesis, photorespiration, and respiration in chloroplast and mitochondria (Foyer *et al.* 2003). Under drought stress, ROS accumulates when its production outweighs removal (Agarwal *et al.* 2005). The over-produced ROS attacks cellular components including nucleic acids, protein, and lipid and eventually leading to cell death (Mittler 2002). ROS scavenging enzymatic activities of superoxide dismutase, catalase, and glutathione peroxidase have been found to increase in 5 soybean germplasms under drought stress (Masoumi *et al.* 2010). Ectopic expression of the *GmPAP3* gene (encoding a mitochondria localized purple acid phosphatase) from soybean significantly reduces ROS accumulation and thereby alleviates osmotic stress (Li *et al.* 2008). Drought stress can also cause misfolding of ER proteins resulting in unfolded protein response (Liu *et al.* 2010). A number of genes have been identified as potential candidates for integration of ER stress signaling responses by global expression-profiling analyses on soybean leaves (Irsigler *et al.* 2007). Moreover, over expression of soybean BiP (binding protein); an ER-resident molecular chaperone enhanced drought tolerance in soybean (Valente *et al.* 2009).

4. Interaction of AMF with other plant growth promoting rhizobacteria (PGPR)

Plant Growth Promoting Rhizobacteria (PGPR) have important role in improving soil fertility. Soil surrounding the plant roots, i.e., rhizosphere is an ecological niche which is integral to many biochemical reactions and is deeply influenced by the root exudates. Here, PGPR play a critical role in plant growth promotion or act on pathogens inhibiting plant growth and also take part in microbial degradation of natural or synthetic compounds. PGPR are also known to elicit systemic tolerance to abiotic stresses such as drought stress. Several ecophysiological studies have demonstrated that AMF symbiosis is a key component in helping plants to cope with water stress and in increasing drought resistance. Investigations on a number of host plants and fungal species have shown that mycorrhizal colonization improves drought resistance of the marigold plants as a consequence of enhancing nutritional status, especially P and water status which in turn enhances plant growth and productivity. Further, plants develop their own survival strategies as well to increase tolerance against drought (Bohnert *et al.* 2006).

AMF, a key member of the rhizosphere, form a symbiotic relationship with the host plant and cast a positive effect on its growth and nutrient uptake. Proliferation of AMF symbioses with the host plant can positively be influenced by PGPR through various mechanisms such as increased spore germination and hyphal permeability in plant roots. Although there are evidences that combined interactions between AMF and PGPR can promote plant growth, better understanding of the interactions between AMF and other microorganisms is necessary for maintaining soil fertility and enhancing crop production (Ramasamy *et al.* 2011). Commonly reported plant growth promotion mechanisms by bacteria are the morphological and physiological changes of the root system, increased roots and root hairs that facilitate more nutrients and water absorption. Higher water and nutrient uptake by roots cause improved water status of plants (Wu and Cosgrove, 2000). PGPR inoculation may help to improve crop resistance against abiotic stress conditions. Exopolysaccharides produced by *Pseudomonas mendocina* binds to soil cat ions including Na and reduce the Na available for plant uptake. Glycoprotein (glomalin) produced by AMF can act as an insoluble glue to stabilize soil aggregates. Drought is a major limitation for crop production in rain-fed ecosystems Synergistic effect of co-inoculated bacteria and AMF help in restoring plant growth under drought conditions (Marulanda *et al.* 2009). The use of indigenous drought tolerant *G. intraradices* strain along with native bacterium reduced 42% water requirement for the production of *Retama sphaerocarpa*. AMF have 500% increased shoot fresh weight (SFW) compared to uninoculated control plants. Interestingly, these AMF plants co-inoculated

with *Azospirillum* showed a further increase of 12% in SFW. In drought stressed conditions, combined inoculation of AMF and *Azospirillum* increased SFW by 103% compared to the uninoculated control. Similar results were also observed by Franzini *et al.*, 2010. Combined inoculation of ACC deaminase positive *Pseudomonas putida* and *Gigaspora rosea* showed increased plant growth and improved root architecture. The results also showed that ACC deaminase producing PGPR strain along with AMF can improve the survivability of plants under stressed conditions. PGPR are associated with plant roots and affect plant productivity and immunity; however, recent work by several groups show that PGPR also induce systemic tolerance to drought. (Yang *et al.* 2009). Specific combinations of autochthonous or allochthonous inoculants also contribute to plant drought tolerance by changing proline and antioxidative activities. However, non-inoculated plants have low relative water and nutrients contents; shoot proline accumulation and glutathione reductase activity, but the highest superoxide dismutase activity, stomatal conductance and electrolyte leakage. Microbial activities irrespective of the microbial origin seem to be coordinately functioning in the plant as an adaptive response to modulated water stress tolerance and minimizing the stress damage (Ortiz *et al.* 2015).

5. Agronomic practices favouring AMF for alleviating drought

Various management strategies have been proposed to cope up with drought stress. Agronomic practices include integrated cropping systems mainly involving crop and soil management practices. In order to increase our ability to optimize management of AMF in field situations, there is a need for more basic information on the seasonal variation in the development of AMF in different crop species and how this is influenced by agricultural practices. The development of a diverse AMF population which can adapt to management and environmental changes is likely to be a key factor in improving the sustainability of low input and organic cropping systems. The results of such studies show that the interactions between plants and AMF are complex and their expression for response is strongly dependent on the environmental factors. Therefore there is a prerequisite to understand the environmental and cultural factors for effective management of AMF.

Crop rotation modifies the population of soil microflora, including AMF by changing the availability of soil nutrients and beneficially reducing the deleterious organisms for the benefit of crop health. Agricultural management thus influences

both the presence of AMF and their activity. Organic agriculture is used, here, as a model for low input agriculture systems. Around 60 years ago Sir Albert Howard, a founder of the organic movement suggested that, the presence of an effective AMF symbiosis is essential to plant health. Studies relating to the effects of different composts on spore populations have revealed that both fungal species and the nature of the organic material are likely to be related to the concentration and availability of nutrients. Within crop rotations, colonized roots and hyphae are an important source of inocula for the cropping sequence (Boswell *et al.* 1998). There are lots of results for the so-called 'rotation effect' in addition to the straight nutritional beneficial effects of N-fixation by legumes in rotations. Cook, 1986 has attributed the 'rotation effect' to improved root health, in which AMF may have an important role. Azcon *et al.* (1996) have addressed the beneficial effects of AMF in reducing disease susceptibility. As a result of finding large changes in mycorrhizal communities associated with different rotations under the same soil and climatic conditions, Hendrix *et al.* (1995) suggested a role for AMF in the rotation effect. Another promising effect of combining rotations with low input systems is weed control. There is evidence that tillage can restrict P nutrition early in the season by disrupting the extraradical hyphae. Tillage may cause a shift in AMF and host plant communities (Douds *et al.* 1995). A study on various cropping systems in soybean resulted in significantly higher AMF spore count (Sharma *et al.* 2012). Inclusion of maize in the rotation irrespective of tillage systems showed comparatively higher phosphatase activities.

Traditional mulching involves covering of the field with straw. The mulch can trap moisture and hence retain soil water. The degrading organic mulch also adds humus to the soil and improves the water holding capacity of the soil that will help AMF to grow very efficiently. It will also help plants to grow under stressed environment. In China, plastic mulch has been widely used on soybean inter planted with maize, potato or cucumber. For example, a study conducted in Shouyang County of the Shanxi Province, China suggested that mulching cultivation with hole-sowing or row-sowing techniques can increase soybean yield up to 23.4% and 50.6%, respectively (Guo *et al.* 2007).

6. Methods of producing AMF and its availability

AMF has been found to be effective as it focuses on developing a healthy soil and enables plants to become better attuned to the environment through an improved nutrient absorption. Therefore its availability has become utmost importance. Since AMF are obligate symbionts, require host to complete its life cycle, therefore production under laboratory conditions is still a complex and difficult to practice by most of growers. There are various methods currently being followed to practice.

6.1 On-farm production

Traditionally, the on farm method of inoculum production has been developed in several ways in a number of countries. The on-farm technology is cost-effective and can be easily transferred to farmers (Douds *et al.* 2006; Sharma and Sharma, 2008). In this system, AMF production is done by farmers on their own property, under natural conditions using indigenous or introduced AMF isolates (Douds *et al.* 2008, 2012). Indigenous AMF species seem to be more efficient in some situations as they are locally adapted to the soil conditions (Sreenivassa *et al.* 1992). Commercial AMF produced using these systems are available in several countries; however, the costs associated with the technology of AMF production, including establishment of single cultures of AMF species, shipping and handling, and development of the carrier substrate are to be borne by farmers and nursery owners (Douds *et al.* 2006).

6.2 Substrate based

Another promising method of production of AMF inoculum uses sand and vermiculite irrigated with nutrient solution. Large-scale multiplication of AMF for field applications is generally carried out in substrate-based, substrate-free, and *in vitro* systems (Ijdo *et al.* 2011). Organic amendments added to the substrate can stimulate sporulation of AMF and replace the nutrient solution. The production of spores varies among the tested AMF and according to the organic source added to the substrate. The vermicompost promotes higher sporulation of certain AMF such as *Acaulospora longula* in relation to other AMF species and substrates (Ijdo *et al.* 2011).

6.3 Aeroponic production of AMF

Production of AMF by aeroponic system enables the production of cleaner spores and facilitates uniform nutrition of colonized plants. This system allows for efficient production of AMF free of any physical substrate. The colonized root material can be sheared resulting in inocula with high propagule densities. Aeroponically produced inocula shows tremendous impact on plant growth and health and can become a key aspect for sustainable agriculture (Singh *et al.* 2012). Multiple techniques have been developed in the past for the mass production of AMF. In the present time, *in vitro* cultivation methods such as hydroponic system and root organ culture have been used for the mass production of AMF. These methods not only maintain the quality of AMF propagule but they can also be developed as cost effective methods for the mass propagation of AMF.

6.4 Hydroponic production of AMF

AMF inoculum can be produced hydroponically where by roots of plants supported on a solid medium or structure are submerged in a reservoir of a nutrient solution such as dilute Hoagland's solution or Hewitt's solution with low phosphorus concentration. (Mosse *et al.* 1984) Full-strength Hewitt's solution (Thompson *et al.* 1986) consists of (mg/L) Ca 160, K 156, N 114 (NO₃ 50–100%), S 112 or 240, P 41, Mg 36, Na 246 or 62, Cl 284, Fe 2.8, Mn 0.55, B 0.33, Zn 0.065, Cu 0.015, Mo 0.015, Co 0.015. The roots of the plant grow through the band of support structure or medium into a nutrient reservoir. Air is continuously bubbled throughout the solution. The nutrient solution is changed at regular intervals. In a submerged sand system, it is necessary to change the medium at an interval of 3–4 days (Thompson *et al.* 1986) Distilled or deionized water is added to the reservoir as needed. Nine to ten weeks after transplanting, plant tops are cut and roots recovered from the reservoir, processed as needed, and either used immediately or stored for use at a later time. Alternatively, AMF roots can be produced by growing suitable nurse plants in a sand matrix submerged in a nutrient solution conducive for AMF development (Thompson *et al.* 1986). Fine roots are sampled and examined for AMF colonization. For instance, Macdonald *et al.* (1981) have described a compact autoclavable hydroponic culture system for the production of axenic AMF formed between *Trifolium parviflorum* and *Glomus caledonius*.

6.5 *In-vitro* production of AMF or ROC (Root Organ Culture)

In-vitro culture of AMF was achieved for the first time in the early 1960s (Mosse, 1962). Since then, various pioneering steps were aimed at axenic culturing of AMF, continuous cultures of vigorous ROCs (Ri T-DNA-transformed) have been obtained through transformation of roots by the soil bacterium *A. rhizogenes* or mass scale production of AMF was achieved by root organ culture in small containers (Tiwari and Adholeya, 2003). These are methods that help mass production of roots in a very short span of time. The ROC is an attractive mass multiplication method for providing viable, rapid and pure inocula. Different production systems have been derived from the basic ROC in petri plates. For example, Gadkar *et al.* (2006) further developed a system where a petri plate containing a ROC was used to initiate fungal propagation in a separate compartment filled with sterile expanded clay balls. Recently monoxenic culture of *G. intraradices* with Ri T-DNA transformed roots in two-compartment petri dishes was also achieved. (Sharma *et al.* 2015). A derived plant *in vitro* production system has also achieved and a patent filed (Declerck *et al.* 2009) where each pre-inoculated *in vitro* produced plant (Voets *et al.* 2009) is individually introduced into a sterile growth tube. A nutrient solution

circulates in that closed system flowing on the mycorrhizal roots. A list of commercial formulations of AM inocula produced by either of the above methods is given in Table 2. The most common method being bulk inocula containing a mixture of spores, colonized roots, hyphae and substrate from the pot containers, usually grown in sterilized soil or soil less media. For substrate-host based method, in addition to use of selected AMF, there is a need to optimize the best substrate and its optimal forms and time of production to obtain maximum sporulation and production of AMF.

Table 2: List of commercial sources/products/suppliers of AM inoculants

Company	Country
Symplanta GmbH & Co. Kg	Munich, Germany
Ag Bio Inc., Westminster	Colorado, USA
Accelerator Horticultural Products	Ohio, USA
Bio-Organics Supply,	Camarillo California, USA
Becker –Underwood, Ames	Iowa, USA
Bio Scientific, Inc., Avondale	Arizona, USA
Eco Life Corporation,	Moorpark California, USA
First Fruits Sarasota,	Florida, USA
J.H. Biotech, Inc., Ventura,	California, USA
First Fruits	Sarasota, Florida, USA
J.H. Biotech, Inc.,	Ventura, California, USA
Mikro-Tek Inc.,	Ontario, Canada
Mycorrhizal Applications, Grants Pass	Oregon, USA
Bio Grow TM	North America
Plant health Care, Inc.,	Pennsylvania, USA
Mycor TM VAM Mini Plug TM	North America
Premier Horticulture, Red Hill	Pennsylvania, USA
Premier Tech	Quebec, Canada
Reforestation Technologies, Salinas	California, USA
Roots Inc., Independence	Montana, USA
T & J Enterprises, Spokane	Washington, USA
TIPCO, Inc., Knoxville	Tennessee, USA
Tree of Life Nursery, San Juan Capistrano	California, USA
Tree Pro, West Lafayette	Indiana, USA
Biological Crop Protection Ltd	Kent, UK
Bio-organics	Medillin, Columbia
Biorize	Dijon, France
Central Glass Co., Chemicals Section	Tokyo, Japan
Global Horticare	Lelystad, The Netherlands
Idemitsu Kosan Co.,	Sodegaura, Chile

(Contd.)

MicroBio, Ltd	Royston, Hertz, UK
N-Viron Sdn Bhd	Malaysia
PlantWorks Ltd., Sittingbourne	UK
Triton Umweltschutz GmbH	Bitterfeld, Germany
KCP Sugar and Industries Corporation Ltd	Andra Pradesh, India
Cadila Pharmaceuticals Ltd	Ahmedabad, India
Symbiotic Sciences Pvt Ltd	Gurgaon, Haryana, India
Symbiom (ViaTerra LLC, Jacksonville)	FL, USA

**Source:* Modified from Sharma and Adholeya (2008)

Conclusion

Soybean is nutritionally and economically important crop. Its growth is affected by various environmental changes such as climatic variations, biotic and abiotic stresses like drought stress. Due to drought stress, soybean production is very much affected worldwide. In this chapter, we have explained effects of AM-mediated drought stresses on soybean and the underlying drought responsive mechanisms. The studies pertaining to morphological, physiological, and molecular changes occurring during stress conditions have led to accumulation of considerable information regarding possible methods of overcoming these stresses. Mycorrhizal plants employ various protective mechanisms to counteract drought stress. The accumulated physiological, biochemical, and molecular data based on classical approaches will benefit from the various 'omic' techniques and their combinations. An in-depth investigation using the advanced methodologies could help to elucidate the mechanisms of drought avoidance and/or tolerance induced by AM symbiosis and to discriminate the drought-induced processes of the protective mechanisms regulated by AM symbiosis. Soybean productivity, under drought, can be improved by integrating all technologies and knowledge involved. Furthermore, some key efforts are needed to identify efficient strains of PGPR and AMF, which can enhance tolerance against biotic or abiotic stresses. Many studies have shown large amounts of hyphal biomass and higher indigenous AMF in crop rotations but the combined application of AMF and PGPR are yet to be streamlined. Most importantly AM inoculum production method particularly ROC systems needs critical evaluation and quality checks by third party/stake holders. The methods for checking the quality assurances need to be standardized. Finally, potential commercial formulations need to be subjected to regulatory requirements and quality checks.

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