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Arbuscular Mycorrhizal Fungi: A Potential Tool for Enhancing Crop Productivity in Salt Affected Soil

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ABSTRACT

AMF have been mentioned in several literatures for long time for their beneficial role in crop growth and productivity. However, recently they are talked about for efficient abiotic stress management which received equal attention among researchers. Salinity is such an abiotic stress faced by crop plants that can be minimized by assistance of AMF. In this article, we have discussed the possible mechanism and the identified species of AMF for abatement of salinity stress of plants by citing some suitable examples, apart from the harmful effects in crop plants' functioning due to salinity usually take place. Though there is lots of scope of further research, this illustrative piece of information may generate interest among farmers and other common people.

Highlights

- AM fungi are potential bio-remediation tool to alleviate salt stress in crops grown in saline soil for sustaining crop productivity in an eco-friendly way.

Keywords: Arbuscular Mycorrhizal Fungi (AMF), Salt stress, Salinity, Crop growth

Saline soil is characterized by higher concentration of soluble salts of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Cl^{-}, NO^{3-}, SO_4^{2-} and CO_3^{3-} etc. Sodic soil is dominated by Na^{+} salt and saline-sodic soil that have both high salt of Ca^{2+}, Mg^{2+}, K^{+} as well as Na^{+}. About 7% of the total land area of world (952.2 million ha) is salt affected. Salt affected which also constitutes nearly 33% of the potential arable land. In India, the salt affected soils account for 6.727 million ha (2.1%) of geographical area of the country. Furthermore, patches of salt affected soils are also emerging due to intensive crop cultivation and excessive exploitation of ground water.

Soil health is one of the key indicators of ecosystem functioning and the ecosystem more resilient to changes. The high salt concentrations adversely affect different soil processes and/or soil properties and in turn environmental conditions. Soil salinity pose a major threat to agricultural productivity as it affects the establishment, growth and development of plants leading to significant loss in productivity (Giri *et al.* 2003; Mathur *et al.* 2007). To alleviate salt stress different techniques *viz*. introduction of transgenic salt tolerant crop variety, leaching, tile drainage etc. have already been employed. Although successful, large scale application of these technique is highly-priced and beyond the reach of developing nations (Evelin *et al.* 2009). Therefore, there is a constant need to generate some eco-friendly as well as effective measure to address this issue.

Plants, in their natural environment are colonized both by external and internal microorganisms. Arbuscular mycorrhizal fungi (AMF) are broad-spectrum and non-specific microorganism that are known to colonize 85% of terrestrial plant species including halophytes, hydrophytes and xerophytes (Pal *et al.* 2013). Several studies have reported the ability of plants to be colonized by AMF in saline conditions, salt marshes and at neutral or moderate alkaline soil pH (Garcia and Mendoza, 2007). The soil borne or extra-matrical hyphae of AMF increase effective absorptive surface area of the plant roots resulting in higher nutrient uptake by crop in nutrient-poor soil (Pal *et al*. 2017). Besides improving nutritional status; they are also capable of enhancing physiological processes like water absorption capacity of plants by augmenting root hydraulic conductivity and favorably adjusting the osmotic balance and composition of carbohydrates (Rosendahl and Rosendahl, 1991; Feng *et al*. 2002). Thus, they alleviate the harmful effects of excess salt accumulated in the root (Dixon *et al*. 1993). Therefore, AMF found in environments with high salt concentrations could be a potential tool for bioremediation of salt affected soils and for augmenting crop productivity in an environmentally safe way.

Salinity effects on crops: an overview

High soil salinity impacts the growth of numerous plant species especially glycophytes (salt-sensitive compared to salt-tolerant halophytes species). Most of the agricultural crops fall in the category of glycophyte which exhibit a spectrum of responses under salt stress. Salinity not only decreases the agricultural production of most crops, but also, affects soil physico-chemical properties, and ecological balance of the area leading to low economic returns and soil erosions. The specific effects comprises of the following:

- Excessive accumulation of sodium in cell walls can results into osmotic stress and cell death (Munns, 2002).
- High salt concentration in soil can disturb the nutrient balance in the plant or interfere with the uptake of some nutrients (Blaylock *et al*. 1994; Chakraborty *et al*. 2015).
- Substantial decrease in leaf area, chlorophyll content and stomatal conductance, leading to less efficient photosystem (PS-II) (Netondo *et al*. 2004).
- Significant reduction in phosphorus (P) uptake by plant due to precipitation of phosphate ions as Ca-phosphate (Bano and Fatima 2009; Bhaduri *et al*. 2016).
- Salinity also impedes reproductive development by impairing micro-sporogenesis and stamen filament elongation, ovule abortion and senescence of fertilized embryos.
- Retarded supply of photosynthetic assimilates or hormones to the growing tissues (Ashraf, 2004).
- Soil salinity also results in ion toxicity where ratio of K^+ to Na^+ changes leading to replacement of K^+ by Na^+ in biochemical reactions. Because of this several enzymatic activities as well as binding tRNA to ribosomes consequently protein synthesis where K^+ is indispensable are inhibited. Moreover high level of Na^+ and Cl^- induced conformational changes in proteins. Recent reports also show that salinity adversely affects plant growth and development by hindering enzyme activity (Seckin *et al.* 2009), DNA, RNA, protein synthesis and mitosis (Tabur and Demir, 2010; Javid *et al.* 2011)
- Ion toxicity and osmotic stress could also cause metabolic imbalance, which in turn leads to oxidative stress (Chinnusamy *et al.* 2006; Chakraborty *et al.* 2016).
- Activities of cyclin and cyclin-dependent kinases are reported to be hindered by soil salinity resulting in fewer cells in the meristem, and limited growth. The activity of cyclin-dependent kinase is diminished also by post-translational inhibition during salt stress.

Role of AMF towards crop growth and nutrition

Interaction between plants and the AMF has a special relevance from ecological point of view, as this association brings about array of physiological changes *viz*. changes in concentration of growth regulating substances, increased leaf area and enhanced photosynthetic rate, photosynthetic partitioning to shoot and roots, improved nutritional status of host tissues facilitating the sustenance, growth and development of plant in the nature. There are number of prioritized issues in sustainable crop production like plant growth and nutrition, soil structuring, stress tolerance and survival, and increasing nutrient use efficiency etc. that have often been achieved by successful intervention of AMF (Yadav *et al.* 2017). The major effects of AM fungi symbiosis on crop growth are broadly as follows:

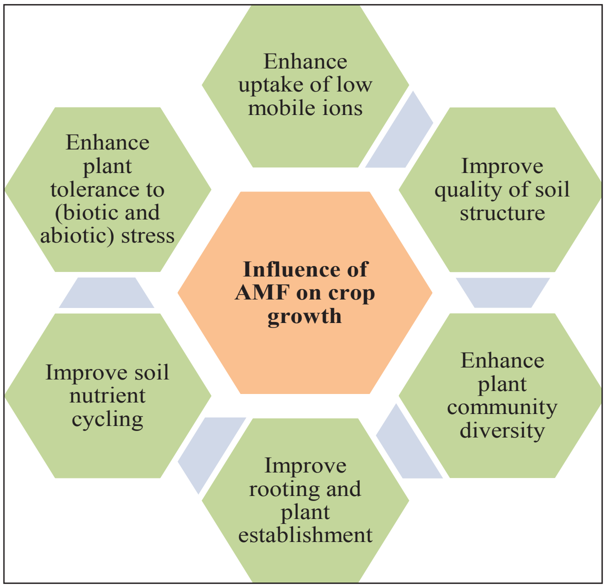

Fig. 1: The major effects of AMF symbiosis on crop growth

Plants, in their natural environment are colonized a wide array of both external and internal microorganisms. Arbuscular mycorrhizal fungi (AMF) are omnipresent and very common among the soil microorganisms dwelling in the rhizosphere and colonizes wide group o plant species in nature. AMF associations with plants are comprised of three main structures; viz: hyphae that performs as extended root surface scavenging the nutrients from the zone beyond the reach of plant roots (Hodge 2000); the second one is vesicles within the root, acting as storage organs, especially for lipids (Hirsch and Kapulnik 1998) and the third one is arbuscules which are branched intercellular structures and works as interface for phosphorus and other nutrient exchange on the root system (Smith et al. 2000). These fungi, being an important as well as integral component of the natural ecosystem and are found to exist in stressed environments such as saline soils (Giri *et al.* 2003). Although AMF are not uncommon in saline soils, their growth and colonization in plant roots has a fair chance to be affected by the excess of salt concentrations, which generally restrict the microbial growth and activity (Juniper and Abbott, 2006). Over the years, AM symbiosis has not only demonstrated the enhanced plant growth and biomass, but also imparted partial resistance to salinity and in a range of host plants such as maize, clover, tomato, cucumber, and lettuce (Rosendahl and Rosendahl, 1991; Ruiz-Lozano and Azcón, 1996; Al-Karaki *et al.* 2001; Feng *et al.* 2002).

A number of studies from last few decades have been carried out for investigating the role of AMF towards the enhanced crop growth and better nutrition. These kinds of studies varied from field crops to horticultural crops to forest sp. The results mostly indicated that symbiosis between AMF and plant root emerged to be beneficial in terms of expressing the physiological behaviour (photosynthetic efficiency, water use efficiency) of plants and nutrient uptake and accumulation in a better way (Porcel *et al.* 2012) that often reflect in increased plant biomass of the host plants. Altogether it was found that AMF-associated plants sustained their growth and yield, and this effect was more pronounced in salt-stress condition. Visually better response in plant biomass differentiated the positive role of AMF over non-inoculated plants (Sannazzaro *et al.* 2007; Zuccarini and Okurowska, 2008; Hajiboland *et al.* 2010). Moreover, better biomass and physiological activities of wheat with root colonization by *Glomus claroideum* (Beltrano and Ronco 2008) and strawberry plants by *Glomus* sp. (Borkowska 2002) were observed under drought stress as well. Antagonistic relationship between plant parasitic nematode and AMF also showed beneficial effect towards the crop growth by enhancing nutrient uptake (Calvet *et al.* 1995). Hence it can be expected that AMF substantially ameliorate both biotic and abiotic stresses, deploying an adequate vigour of crop plants.

Uptake of essential nutrients like P, K and micronutrients like Fe, Zn, Cu is often researched and highlighted under influence of AMF both under saline and non-saline soil conditions. However, when a plant is already facing a stress and the nutritional balance is already disturbed, the favorable effect created by AMF symbiosis is more pertinent in this regard. It is speculated that higher K^+ accumulation by tissues of mycorrhizal plants under salt stress may help to maintain a high K/Na ratio, which further may help into normalization of various enzymatic processes and protein synthesis (Porcel *et al.* 2012). Similar results showed in *Acacia nilotica* plants when colonized by *Glomus fasciculatum* was found to have a higher K^+ ion concentration in both root and shoot tissues even at all salinity levels (Giri *et al.* 2007). Improvement of P-ion uptake and accumulation in plants by better absorption of hyphal network articulated by AMF had repeatedly been evidenced over the decades (Plenchette and Duponnis, 2005; Sharifi *et al.* 2007).

Ameliorative mechanisms involved in abatement of salt stress in crops

Salt stress can potentially cause a severe setback to plant growth, yield, physiological attributes and nutrient uptake behaviour. However, there are number of studies carried out to assess the role of AMF for alleviating the salt stress in crop plants, and most of them reported a positive outcome. The following figure (Fig. 2) depicts in brief the mechanisms imparted by AMF for abatement of salt stress to crops.

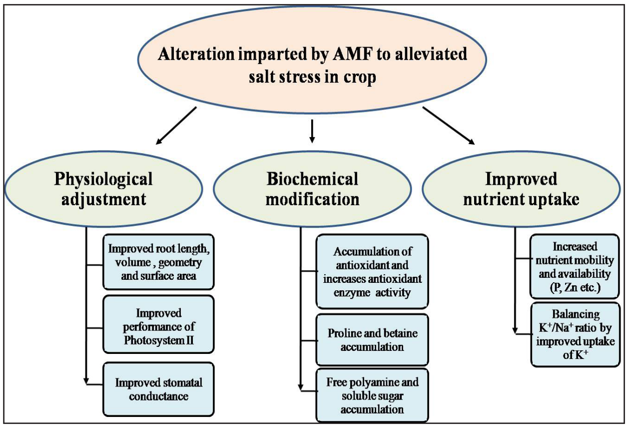

Fig. 2: The ameliorative mechanisms involved in alleviating salt stress in crops

Morpho-physiological changes

Salt stress can potentially cause a severe setback to plant growth, yield, physiological attributes and nutrient uptake behaviour. However, there are number of studies carried out to assess the role of AMF for alleviating the salt stress in crop plants, and most of them reported a positive outcome. Morphological and physiological characteristics of plants are keys to address any abiotic (salt) stress management hence most integral part of such experiments.

Many examples can be found in horticultural crops regarding useful inoculation of AMF. Association of AMF showed beneficial effect on root morphology of *Citrus tangerine* seedlings and enhanced the characters like root length, root projected area, root surface area and root volume under salinity (Wu *et al.* 2010a). Mycorrhizal colonization also caused improvement in fruit fresh weight, fruit number and fruit yield of salt-stressed tomato plants (Al-Karaki, 2000; Latef and Chaoxing, 2011). Improved growth, yield and quality of fruits of *Cucurbita pepo* plants was also noticed when colonized by *Glomus intraradices* in salinity stress (Colla *et al.* 2008). Olive plants inoculated with *Glomus mosseae* helped to survive the plants better in salt stressed condition in terms of enhanced root and shoot growth, and lesser biomass reduction (Porras-Soriano *et al.* 2009). Among field crops, AMF inoculated maize plants showed to have better root morphology (length, mass, surface area, diameter, and volume) under imposition of salt treatments (Sheng *et al.* 2009).

The better performance of photosystem-II and stomatal conductance, and more accumulation of antioxidants of maize plants inoculated with native AMF sp. jointly contributed to enhance salt tolerance (Estrada *et al.* 2013). Chlorophyll content, photosynthesis and leaf water content of peanut plants were found to increase under salinity stress by the inoculation with mycorrhizal fungi (*Glomus mosseae*) (Al-Khaliel 2010). AMF inoculated citrus seedlings showed more contents of ascorbate, soluble protein and glutathione and greater activity of catalase under both saline and non-saline conditions and thus imparted better antioxidant defense system in plants (Wu *et al.* 2010b). On a similar note another group of worker also evidenced that AMF may protect tomato plants against salinity by alleviating the salt induced oxidative stress by increasing of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase and reduced malon-di-aldehyde (MDA) content (Latef and Chaoxing, 2011).

Biochemical alterations

Plant tolerance to salinity is a complex trait to which plants have evolved responses involving biochemical and molecular mechanisms. Additional plant responses can include selective build-up or exclusion of salt ions, maintenance of photosynthesis at values adequate for plant growth, changes in membrane structure and synthesis of phytohormones (Turkan and Demiral 2009)

AMF are reported to improve tolerance and/or resistance of plants against salinity by increasing accumulation of these compounds.

Proline, betaine accumulation in plant is the most studied modifications following salt exposure.

Proline and betaine accumulation has been found to increase when the *Glycine max* is colonized by *Glomus etunicatum* (Sharifi *et al.* 2007) and *Phragmites australis*is colonized by *Glomus fasiculatum* (Al-Garni 2006).

Free polyamines mainly putrescine, spermidine and spermine are recognized as a key role in plant responses to a wide array of environmental stressors such as salinity, osmolarity and antioxidative stress (Evelin *et al.* 2009). They are essential in regulating root development under salt exposure (Couee *et al.* 2004). Level of free polyamine pools is declined under salt stress condition; nevertheless, the inoculation of host plants with AMF increases free polyamine concentrations (Sannazzaro *et al.* 2007).

Abscisic acid (ABA), a phytohormone, is well known for its signalling role in the regulation of plant growth and development in response to the salinity stress by closing the stomata to reduce water loss and inducing the expression of stress-related genes that reduces the adverse effect it has caused (Evelin *et al.* 2009).

Another plant tolerance mechanism involves accumulation of soluble sugars that lowered down the osmotic potential of the plant (Thanna and Nawar 1994). Plants (*Phragmites australis*, *Glycine max*) colonized by AMF (*Glomus fasciculatum*, *Glomus intraradices*) are reported to have higher level of soluble sugars than those non-mycorrhizal plants (Porcel and Ruiz-Lozano 2004; Al-Garni 2006). Moreover, Trehalose, is a non-reducing disaccharide acts as the prime storage carbohydrate in AMF. It functions as abiotic stress protectant that stabilizes dehydrated enzymes and membranes, safeguards biological structures from desiccation damage. Although, presence of trehalose is very infrequent in higher plants, but it gets induced by AMF colonization of plant roots, in turn protects plants against salt stress (Hoekstra *et al.* 1992; Schubert *et al.* 1992).

Plants with high concentrations of antioxidants have been perceived to impart greater resistance against oxidative damage (Jiang and Zhang 2002). Most common antioxidant enzymes are superoxide dismutase, catalase, dehydroascorbate reductase, glutathione reductase and different peroxidase. Plants having their roots colonized by AMF are reported to abate salinity stress through augmenting the activities of antioxidant enzymes (Ghorbanli *et al.* 2004; Zhong Qun *et al.* 2007).

Chlorophyll content in leaves is another parameter which could differentiate between mycorrhizal and non-mycorrhizal plants exposed to salt stress. AMF colonization is reported to reduce salt interference in chlorophyll synthesis (Giri and Mukerji 2004; Zuccarini 2007; Colla *et al.* 2008; Sheng *et al.* 2008) as well as increases chlorophyll content in *Capsicum annuum, Glycine max, Cyamopsis tetragonoloba* and *Zea mays* (Beltrano *et al.* 2013; Datta and Kulkarni 2014) Furthermore, the antagonistic effect of Na^+ ion on Mg^{2+} uptake is counter balanced and suppressed (Giri *et al.* 2003) in the presence of mycorrhiza. Chlorophyll fluorescence, a measure of photosynthetic efficiency is calculated as the ratio between variable and maximum fluorescence (Fv/Fm) which indicates capacity of the primary photochemistry of PSII (Sheng *et al.* 2008; Zuccarini and Okurowska 2008). The ratio Fv:Fm is very sensitive to salt stress. Nevertheless, mycorrhiza helps plants to maintain significantly higher Fv:Fm ratio thus maintaining the photosynthetic efficiency under saline stress condition (Sheng *et al.* 2008; Zuccarini and Okurowska 2008).

Various studies have also documented that AMF symbiosis helps plants to alleviate salt- or water-deficit stresses by enhancing the activities of antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase, monodehydroascorbate reductase, guaiacol peroxidase, oxidized glutathione, glutathione peroxidase (Zhong *et al.* 2007; Garg and Manchanda 2009; Talaat and Shawky 2011). Thus, mycorrhizal plants possess enhanced activity of several antioxidant enzymes.

Improved nutrient uptake

Facilitating the nutrient uptake for plants by use mycorrhiza has repeatedly been highlighted by the researchers throughout the world. Apart from primary soil nutrients (N, P, K), AMF proved its efficiency to absorb Mg, Ca, Cu, Zn, Fe, Ni, Cd through plant roots. It is thus often been considered that uptake and transport of nutrients from soil is the primary function of mycorrhizal fungi associated with plant roots (reviewed in Quilambo 2003). The fungal hyphal network is ideally positioned to efficiently take up nutrients and water from soil, but only a few fungal transporters are involved in this process, including those that transport phosphate, ammonium and zinc. Because of diffusion is too slow the nutrients are moved in a packaged form between the extra-radicle and the intra-radicle mycelium. Possible mechanisms of nutrient uptake are:

(a) Better absorption of nutrients: The mycorrhizal plants absorbs the nutrients more efficiently compared to non-mycorrhizal ones as AMF changes root space geometry and increases root surface area, acts as absorbing surface, increases the efficiency of roots to absorb nutrients and also enhances the life span of roots keeping them active for greater period.

(b) Increasing nutrient availability: Some AMF synthesize phosphatises which increase mineralization of organic phosphate and increase phosphate availability while few AMF produce organic acids which increases pH and in turn solubility and availability of phosphate.

(c) Increasing availability: AMF may also solubilize some unavailable form of mineral phosphate to available form enhancing its availability.

Phosphorus absorption has often been easier under mycorrhizal inoculation; even under saline soil the P uptake was found higher (Tian *et al.* 2004; Sharifi *et al.* 2007; Al-Khaliel 2010). P, being a poorly mobile nutrient as PO_4^{-3}, when show a positive influence in presence of AMF towards absorption in plant roots, and that also under problem (saline) soil, demands a special mention. Despite P, N and K uptake were also found improved by association of AMF (Rabie and Almadini 2005; Al-Khaliel 2010). Garg and Manchanda (2008) and Giri and Mukerji (2004) both reported a higher N uptake in presence of *Glomus* sp. under soil salinity by *Cajanus cajan* and *Sesbania* sp. respectively. Enhancement of potassium uptake under salt-stressed soil was also found for Soybean plants (Sharifi *et al.* 2007).

A selective uptake of nutrients is also sometimes mentioned by AMF. Balancing the K^+/Na^+ ratio in plant tissues is a major concern to avoid the deleterious effects of soil salinity. AMF was also found to interfere in the increased uptake of K with concomitant decreased uptake of Na by plant roots (Zuccarini and Okurowska 2008). Ca^{+2} and Mg^{+2} were also found to absorb more by plant roots with myccorrhizal association despite of soil salinity (Yano-Melo *et al.* 2003; Sharifi *et al*. 2007; Giri and Mukerji 2004).

Negative effect of salinity can also be alleviated by AM-fungi due to reduced uptake of Ca and K and increase in levels of P, Zn and Cu in leaves (Ezz and Nawar 1993).AM fungi improved plant tolerance to salinity through better host plant nutrition, and maintained higher K^+/ Na^+ ratio in plant tissues (Estrada *et al.* 2013).

This sort of beneficial activities by AMF is often enhances the nutrient use efficiency of plants in marginal or degraded soils, depleted with the essential nutrients. This may further aggravate the soil fertility and productivity.

Potential genera of AMF

Arbuscular mycorrhizal fungi have been reported to occur naturally in saline environments. The most commonly observed genera in saline soils is Glomus spp. belonging to family Glomeromycota, however, the ecological specificity has not yet been demonstrated. Studies of early dates employed only morphological characterization to identify the AMF spores. Nevertheless, fatty acid methyl ester (FAME) study, use of molecular techniques such as polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) are aiding better and more accurate enumeration of the AM fungal diversity in saline soil area. The dominant genera of AM fungi having potential of salt stress amelioration are mainly *Glomus intraradices*, G. *versiform*, G. *Etunicatum* (Aliasgharzadeh *et al*. 2001; Porras-Soriano *et al*. 2009) G. *fasciculatum*, G. *macrocarpum*, G. *geosporum*, G. *coronatum* (Giri and Mukerji 2004; *Giri et al. 2007*), *Gigaspora gigantean*, G. *margarita* (Sambandan 2014), *Acaulospora*, *Archaeospora* (Wang *et al*. 2004), *Funneliformis mosseae*, F. *geosporum*, F.*coronatum*, *Rhizofagus fasciculatus* (Bencherif *et al.* 2015).

Crop productivity enhancement through AM Fungi intervention in salt affected soils

AMF with their favourable symbiotic relationship with plants could also play a crucial role in enhancing crop productivity in salt affected soil.

Table 1: Potential genera of arbuscularmycorrhizal fungi reported for combating salinity stress

Sl. No	Potential Genera	Plant growth benefit
1	*Glomus intraradices, Glomus mosseae*	Improved growth, yield and quality of fruits in Pumpkin.
	Glomus mosseae	Enhanced root and shoot growth and lesser biomass reduction in olive plant. In maize plants imparted better root morphology (length, mass, surface area, diameter and volume).
3	*Glomus mosseae, Glomus fasciculatum*	Increased Proline content in Maize, Soybean
4	*Glomus mosseae, Glomus fasciculatum, Glomus clarum*	Increased soluble carbohydrate content in Green gram, Soybean.
5.	*Glomus intraradice, Glomus etunicatum*	Increased Abscisic acid in Lettuce and Tomato.
6.	*Glomus mosseae, Glomus versiforme, Glomus intraradices, Glomus etunicatum*	Increased antioxidant enzyme activity in tomato and cowpea.

With the help of their extra-matrical hyphae they increase the absorptive surface area of the roots of the host plants leading to more accessibility and absorption of relatively immobile elements in soil such as P, Cu and Zn (Menge and Timmer 1982). Till date, only few studies have reported the role of AMF in direct crop productivity enhancement. Tripartite association among AMF, *Bradyrhizobium* and mungbean (*Vigna radiate* L.) greatly helped in establishment and cultivation of the crop in the saline soils of West Bengal, India (Singh *et al.*, 2011). Daei *et al.* (2009) studied the influence of *Glomus etunicatum*, *G. mosseae*, *G. intraradices* on enhancing wheat growth under salinity, where the electrical conductivity of the irrigation water was as high as 13.87 dS/m. The increase in grain yield ranged from 4.5 to 38.3% mainly due to *Glomus etunicatum* and *G. intraradices*, respectively. *Glomus etunicatum* appeared to be the best performer among three species. This reflected the great significance of choosing the right combination of AM species and host plant to exploit the symbiosis benefit on crop productivity under salinity condition. Similarly, a study on influence of combined application of mycorrhizal fungi and compost reported improved berseem yield owing to increased uptake of nutrients (P, Mn, Zn, Fe, Cu) in alkaline calcareous soil (Jan *et al.* 2014).

CONCLUSION

Role of AMF in abating salt stress on crop growth is still unexplored. Thus, ample research opportunity is there to screen out the suitable location-specific as well as crop-specific AM fungal strains. The most efficient AM fungal strain needs to be exploited for enhanced productivity under salinity-prone areas. In this connection, the emerging strains can be studied for identifying their various morphological, and biochemical effects on plants. The newly identified strains can also be tested for facilitated uptake of single or multiple nutrients ions through plant cells. Moreover, studies on AMF diversity as well as signaling mechanism, prerequisite for host plant colonization by AM fungal species, are also necessary for understanding the ecological function played by AMF under wide range of salt affected condition. Further the potentiality of AMF can be tested for multiple abiotic soil stresses along with salinity.

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