

## Review

## Myth and mystery of soil mycorrhiza: A review

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Arbuscular mycorrhizal (AM) fungi are soil borne fungi forming symbiotic relationship with majority of higher plants providing a direct link between soil and plant roots. AM fungi undergo a multi-step colonization process before benefiting the host plant. The strong glue glomalin is a glyco protein produced by an AM fungi. The deposition of glomalin on soil particles leads to buildup and stabilization of aggregates, which leads to better soil structure which in turn leads to increased soil water availability under water stress condition. The mycorrhizal colonization is effective aggregators and therefore management of mycorrhizal fungi can be considered as a biological amendment for soil structure and other soil physical properties. Mycorrhizal plants could take up more metal nutrients via extraradical hyphae, which provide larger surface areas than the roots alone and reduce the distance for diffusion, thereby enhancing the absorption of immobile metal nutrients (especially Phosphorus and Zinc). Mycorrhizal inoculated plants produce larger biomass as a direct consequence of improved photosynthetic activities and translocate 20 to 30% of the assimilated C to the underground where mycorrhizal structures conserve soil carbon. AM symbiosis enhanced the plant growth by increasing plant access to immobile mineral ions mainly Phosphorus and Zinc, improving physical conditions and by binding heavy metals into roots that restricts their translocation into shoot tissues. The mycorrhizal symbiosis assists in biofortification of micronutrients such as Fe and Zn in maize grain irrespective of calcareous and non-calcareous soils. The mechanism involved in improved micro nutrition of maize includes acidification of rhizosphere; siderophore production enhanced physiologically active Fe and production of anti-oxidants besides synergistic interaction between P and micronutrients.

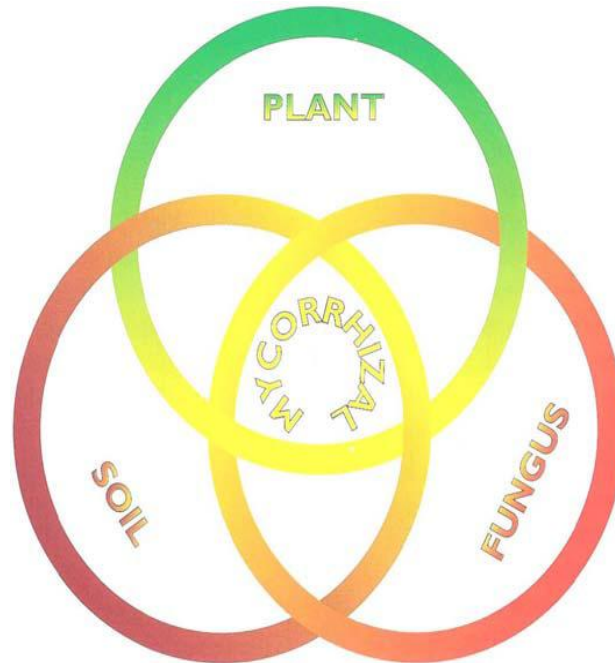
**Key words:** Mycorrhiza, soil aggregation, micronutrients, carbon sequestration, heavy metals, enzymatic activity, glomalin, biofortification.

### INTRODUCTION

The term mycorrhiza is rooted from two Greek words myco meaning fungi and Rhiza meaning root and its' meaning in reality means symbiosis between a fungus and root. Mycorrhiza is described as a mutual sharing of life; whereby the fungal is the major partner of the plant

has the duty to supply food, growth hormones and protection of plants' root from pathogens and a fine plant will offer high energetic material to the fungus (Alizadeh, 2011). Arbuscular mycorrhizal fungi (AMF) play an important role in vegetation restoration because of

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**Figure 1.** Mycorrhizal association, showing the interactions between fungus, plant and soil (Brundrett et al., 1996).

symbiosis with plant root; they can facilitate mineral absorption by the host plant, stabilize and improve soil structure, affect the population structure and preserve species diversity (Bothe et al., 2010). They are key components of the soil biota and account for about 25% of agricultural soil microbial biomass. The major types of mycorrhizae are ecto and endo mycorrhizae. The ecto mycorrhizae are characterized by an extra cellular fungal growth in the root cortex. The endo mycorrhizae are characterized by inter and intra cellular fungal growth in the root cortex. The largest group of endo mycorrhizae is vesicular arbuscular mycorrhizae. Now, it is called arbuscular mycorrhiza (AM).

AM fungi exist in soil as chlamydospores or as vegetative propagules in roots that germinate in rhizosphere. Their hyphae penetrate the root cortex, ramifying intracellularly from the point of penetration. The fungus forms haustoria like structure called arbuscule within cortex cells, where the metabolites exchange between the fungus and the host plant takes place. The other structures are vesicles that are lipid storage and reproductive structures present in the intra cellular spaces. AM fungi transport water and mineral nutrients from the soil to the plant while the fungus is benefiting from the C compounds provided by the host (Figure 1).

Among the mycorrhizal associations, vesicular arbuscular mycorrhizae (VAM) are most widespread in their distribution both geographically and among plant species. The Glomeromycota consists of fungi that are generally considered to be obligately symbiotic. Although

probably correct, this is an assumption based on analogy with the species for which the biology is known. Such species have been shown either (in one instance) to have a Nostoc (Cyanobacteria) species as a symbiont (Schüßler, 2002), or (in all other known instances) to form an intimate symbiosis, generally known as an arbuscular mycorrhiza (AM), with embryophytes (land plants). Vascular land plants, hornworts (Schüßler, 2000) and liverworts (Fonseca and Berbera, 2008) may also form AM. Many glomeromycotan species are known to form AM, but many others have been described from field collected specimens for which the nutritional state of the fungus is unknown.

### MYCORRHIZAS ON SOIL AGGREGATION

Rillig and Mummey (2006) reviewed the contribution of AMF to soil structure at various hierarchical levels. Stevens et al. (2010) found alter AMF prevalence in wetlands could significantly alter plant community structure by directly affecting seedling growth and development. The strong glue glomalin is a glyco protein produced by an AM fungi. The deposition of glomalin on soil particles leads to buildup and stabilization of aggregates, which leads to better soil structure which in turn leads to increased soil water availability under water stress condition. Since glomalin contains 30 to 40% C containing compounds that protect the soil from drying out. Pools of organic carbon produced by AM fungi may

**Table 1.** Positive effects of VAM in nutrient's absorption.

Nutrient	References
Phosphorus	Harley and Smith (1983), Al-Karaki and Al-Radad (1997), Chandreshekara et al. (1995)
Nitrogen	Liu et al. (2002)
Potassium	Liu et al. (2002)
Magnesium	Gildon and Tinker (1983), Li et al. (1991)
Copper	Faber et al. (1990), Gildon and Tinker (1983)
Zinc	Chen et al. (2003), Jamal et al. (2002)
Calcium	Liu et al. (2002)
Iron	Caris et al. (1998)
Cadmium	Guo et al. (1996) Gonzalez et al. (2002)
Nickel	Jamal et al. (2002), Guo et al. (1996)
Uranium	Rufyikiri et al. (2002)

even exceed soil microbial biomass by a factor of 10 to 20 (Rillig et al., 2001). The persistence of soil hyphae also makes arbuscular mycorrhizal fungi important in longer term aggregate stabilization and in the formation of water stable aggregates. Fungal hyphae are known as extra radical hyphae acquire nutrients, propagate the association and produce spores and other structures. The fungal hyphae grow into the soil matrix to create the skeletal structure that holds primary soil particles together via physical entanglement. They create conducive conditions to the formation of micro aggregates and smaller macro aggregates into macro aggregate structures.

The mycorrhizal hyphae colonization is effective aggregators and therefore, management of mycorrhizal fungi can be considered as a biological technique for improving soil structure. External to the roots, mycorrhizal fungi can alter the chemical and physical properties of soil due to the effects of the extraradical hyphae (Smith and Read, 1997). These hyphal associates frequently produce sticky material that causes soil particles to adhere and form aggregates. The physical entanglement of external mycelium of mycorrhizal fungi has been proposed as the major soil aggregating mechanism. Soil structure dictates the water and gaseous movement in soils which eventually affects the soil moisture depletion pattern. Subramanian et al. (1997) have shown that mycorrhiza colonized soil retained higher moisture content throughout the three weeks of drought cycles despite greater depletion by the larger dry mass of the host plant. Thus, mycorrhizal colonization improves physical conditions of the colonized soils

### MYCORRHIZAS ON NUTRIENT UPTAKE

Under normal condition, the concentration of available P is very low and accounts for only 1.55% of

total P content. Because P is an extremely immobile nutrient in soils even if P was added to the soil in soluble form, soon it becomes immobilized as organic P and most of the inorganic P is adsorbed on to the soil surfaces or precipitated as Fe and Al phosphate in acid soil and Ca and Mg in alkaline and calcareous soil. Effects of VAM in nutrient's absorption were given in Table 1. AM play a vital role in increasing the P uptake by the following mechanisms:

- i. Through exploration of soil volume making positionally unavailable nutrients available. This is achieved by decreasing the diffusion of phosphate ions and by increasing the surface area for absorption. The diameter of root hairs is commonly larger than 10  $\mu\text{m}$  compared to 2 to 4  $\mu\text{m}$  diameter of hyphae and has more surface area for greater absorption of nutrients.
- ii. Faster movement of P in to mycorrhizal hyphae is achieved by increasing the affinity for P ions and by decreasing threshold concentration required for adsorption of P.
- iii. AM produces oxalic acid and phosphatase enzyme which has high complexation constants for Ca, Fe and Al solubilize considerable amounts of P. Besides, increasing the P and N uptake, the micronutrient especially Zn and Cu uptake was also increased by mycorrhizae (Suri et al., 2011).

### MYCORRHIZAS ON MICRONUTRIENTS AVAILABILITY

Arbuscular mycorrhiza inoculation has also been shown to improve growth and Zn nutrition of wheat and maize in Zn-deficient soils, due to better access of roots to native Zn and Zn added as fertilizer (Kothari et al., 1991). The mobility of Cu, Zn, Mn and Fe in soils is low. As a result, uptake of these nutrients by roots is limited (Barea, 1991;

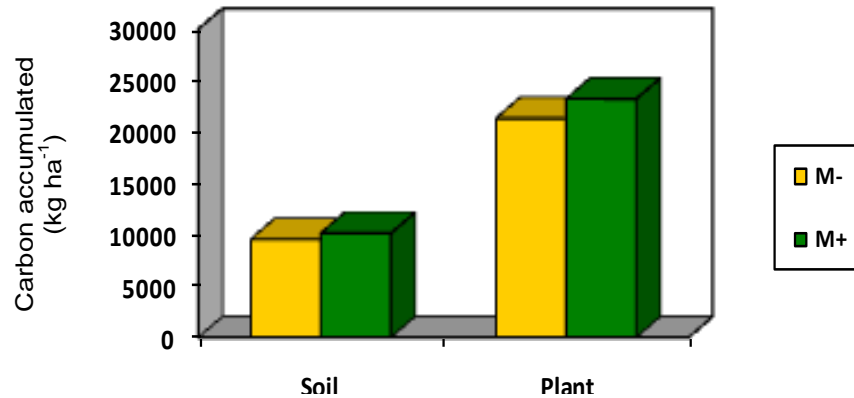


Figure 2. Carbon stock in below ground and above ground (Subramanian et al., 2011).

Tisdale et al., 1995). When no micronutrients were added to the soil, available Cu, Zn, Mn and Fe levels were low and a depleted zone of these nutrients would have formed around the roots. As a result, the uptake of these nutrients was limited in non-mycorrhizal plants. Mycorrhizal plants could take up more metal nutrients via extraradical hyphae, which provide larger surface areas than the roots alone and reduce the distance for diffusion, thereby enhancing the absorption of immobile metal nutrients (Jakobsen et al., 1992). Arbuscular mycorrhizal (M+) fungi are widespread and agronomically important plant symbiont and often stimulate plant uptake of nutrients such as P, Zn, Cu, and Fe in deficient soils (Liu et al., 2000) and increases resistance of plants to heavy metals and salts (Colla et al., 2008; Subramanian et al., 2008).

### MYCORRHIZAS ON CARBON SEQUESTRATION

Major part of research into biological effects of rising atmospheric CO<sub>2</sub> concentrations and temperature has focused on plant growth and carbon fixation. However, the crucial component of terrestrial ecosystems, especially in the soil, has received less attention. Arbuscular Mycorrhizal (AM) fungi form symbiotic association with more than 90% of terrestrial plant species and are known to improve nutritional status of host plants besides providing tolerance to abiotic and biotic stress conditions. The host plants have the ability to control the C flow into the soil where it may get accumulated or be oxidized to the atmosphere as well as nutrient flow to the host plants and thereby indirectly controlling the photosynthetic activities of host plants. Another important consequence of carbon flow to the fungal partner is the development of a unique rhizosphere microbial community called the mycorrhizosphere, which will be discussed shortly. Soil scientists now realize that carbon flow to the soil is critical for the development of soil aggregation and the

maintenance of a healthy plant-soil system. The net verifiable carbon accumulation in mycorrhizal soil was 10,140 kg ha<sup>-1</sup> while uninoculated soil had just 9,555 kg C ha<sup>-1</sup> with a net gain of 585 kg C ha<sup>-1</sup> (Figure 2).

The total above ground biomass of mycorrhizal treatment was 23,215 kg C ha<sup>-1</sup> higher than M- treatment (21,318 kg C ha<sup>-1</sup>) indicating excessive amount of C approximately 2000 kg C ha<sup>-1</sup> gets sequestered in the presence of mycorrhizal inoculation (Subramanian et al., 2011). Enhanced carbon flow to the soil should be considered an important benefit of mycorrhizal colonization under changing climate.

Overall, the data strongly suggest that there is an enormous potential for mycorrhizal fungi to conserve soil C that is yet to be explored in depth. A challenge in mycorrhizal research is to gain insights into the C flows in mycorrhizal system and relate how these processes will contribute for the emission of CO<sub>2</sub> and carbon balance in the soils. The sequestration of soil carbon in organic matter involves the production of complex organic structures, biomass carbon and assimilation by vegetation. Forests are major reservoir of terrestrial above-ground C which is estimated as 66% composed of 44% in forests and 12% in agro-ecosystems (Dixon et al., 1994).

### MYCORRHIZAS ON HEAVY METALS

The AM fungal surface area serves as adsorptive sites for heavy metal cations and this process prevents entry of toxic metals into the host plants (Joner et al., 2000). The binding of metals takes place on the fungal wall through electrostatic interactions. The heavy metal ions in soil solution interact with mycorrhizal cell wall which is rich in negatively charged ligands such as phosphoryl, carboxyl, hydroxyl and phenolic groups. Christie et al. (2004) indicated that the external mycelium of *Glomus mosseae* bound more than 3% of Zn on a dry weight basis which can be considered as a potential mechanism

**Table 2.** Interactions of Arbuscular Mycorrhizas with beneficial soil microorganisms (Azcbn-Aguilar and Barea, 1997).

Type of microorganism	Results of the interaction
N,-fixing bacteria (biofertilizers)	N, fixation, N-cycling, N 'transfer'
Phosphate solubilizers (biofertilizers)	P-cycling, use of reek and organic phosphates as an alternative P source
Plant hormone producers (phytostimulators)	Rooting and establishment of seedlings
Agents for biological control of plant diseases ('biopesticides', bioprotectors)	Increased resistance/tolerance to root diseases
Bacteria and fungi related to formation of stable aggregates (ecosystem bioremediators)	Improvement of soil quality

in alleviating Zn phytotoxicity in contaminated sites. Joner et al. (2000) reported that *G. mosseae* P2 isolated from contaminated sites accumulated four times of Cd (2000  $\mu\text{mol/g}$ ) in comparison to *G. mosseae* (450  $\mu\text{mol/g}$ ) in unpolluted soils.

Mycorrhizal association is known to improve the host plant tolerance to nutrient deficiencies (Smith and Read, 1997; Jeffries and Barea, 2003), drought (Nelson, 1987; Augé, 2001; Subrmanian et al., 2006), chilling (Charest et al., 1993), salinity (Ruiz-Lozano et al., 1996; Azcon and El-Atrach, 1997), heavy metals (Joner et al., 2000; Gaur and Adholeya, 2004; Audet and Charest, 2006) and root feeding pathogens (Linderman, 1994; Benhamou et al., 1994). The role of AM fungi is utmost important in heavy metal contaminated soils where the biological activity is absolutely nil and nutritional status is extremely poor. Even under degraded soils, AM symbiosis can contribute to plant growth by increasing plant access to immobile mineral ions such as P (Vivas et al., 2003; Yao et al., 2003), improving physical conditions (Rillig and Steinberg, 2002; Steinberg and Rillig, 2003) and by binding heavy metals into roots that restricts their translocation into shoot tissues (Dehn and Schuepp, 1989; Kaldorf et al., 1999). Thus, there is an immense potential for mycorrhizal symbiosis in revegetation of contaminated soils. Even though AM fungi are omnipresent in terrestrial ecosystems, chemical pollutants in the soils can substantially reduce fungal population and their functionalities. The number of fungal spores and root colonization of plants are often reduced by soil disturbance (Waaland and Allen, 1987). However, some indigenous AM fungal species would have inherently developed built-in mechanisms that assist in adaptation to hostile environment (Gaur and Adholeya, 2004).

### MYCORRHIZAS ON SOIL MICROBIAL POPULATION

Soil is a complex and dynamic environment in which the

biological activity is mostly governed by microorganisms and organic matter. In the plant rhizosphere root exudation and rhizodeposition stimulates the microbial community. AMF are probably the most abundant fungi in agricultural soil accounting for somewhere between 5 and 50% of biomass of soil microbes live on carbohydrates obtained from the root cells. They alter root exudation considerably (Marschner et al., 1997) and are therefore expected to influence rhizosphere populations as well (Hayman, 1983). Numerous studies have shown conclusively that AM is having synergistic interaction with other beneficial soil microorganism such as N fixers and P solubilizers. AM fungi affect the composition of bacterial communities either directly by changing host plant physiology or indirectly by changing the pattern of root exudation.

The interactions of Arbuscular Mycorrhizas with beneficial soil microorganisms is showed in Table 2 (Azcbn-Aguilar and Barea, 1997). Zhang et al. (2008) examined microbial community diversity of mycorrhizospheres in five tree species, *Caragana microphylla* L., *Hippophae rhamnoides* L., *Sophora viciifolia*, *Pinus tabulaeformis* Carr. and *Robinia pseudoacacia*. The results demonstrated that the different tree species differ significantly in both mycorrhizal infection rates and microbial functional diversity. Zhang et al. (2010) revealed that the AMF and bacterial Shannon diversity index in the rhizosphere of *H. rhamnoides* was higher than that of *Caragana korshinkii* and AMF communities had a significant positive correlation with the bacterial communities in Zhifanggou watershed on the Loess Plateau.

### MYCORRHIZAS ON ENZYMATIC ACTIVITY OF SOIL

Soil enzymatic activities have been described to establish indices of soil fertility, soil productivity and soil quality (Busto and Perez-Mateos, 1997). There have been many

**Table 3.** Influence of mycorrhizal inoculation on dehydrogenase ( $\Delta$  in OD at 485 nm) at 45 and 75 DAS under Fe-Zn interactions (Mean of 4 replications).

Treatments	Calcareous								Non-calcareous							
	Sterilized soil				Natural soil				Sterilized soil				Natural soil			
	45 DAS		75 DAS		45 DAS		75 DAS		45 DAS		75 DAS		45 DAS		75 DAS	
	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>
Fe <sub>1</sub> Zn <sub>1</sub>	0.112	0.196	0.210	0.286	0.120	0.223	0.262	0.382	0.132	0.274	0.192	0.274	0.206	0.298	0.210	0.352
Fe <sub>2</sub> Zn <sub>1</sub>	0.096	0.128	0.213	0.294	0.140	0.245	0.275	0.386	0.223	0.282	0.210	0.281	0.215	0.330	0.214	0.383
Fe <sub>1</sub> Zn <sub>2</sub>	0.120	0.219	0.224	0.334	0.162	0.247	0.296	0.394	0.245	0.286	0.214	0.279	0.224	0.344	0.233	0.362
Fe <sub>2</sub> Zn <sub>2</sub>	0.143	0.240	0.222	0.356	0.175	0.287	0.228	0.364	0.219	0.294	0.223	0.313	0.247	0.333	0.218	0.387
Mean	0.118	0.196	0.217	0.318	0.149	0.251	0.265	0.374	0.205	0.284	0.210	0.287	0.223	0.326	0.219	0.371

**ANOVA: M (Mycorrhizal inoculation), F (Fe levels), Z (Zn levels)**

M	0.0064**	0.0013**	0.0064**	0.0013**	0.009**	0.011**	0.012**	0.0013**
F	0.0064**	0.0013**	0.0064**	0.0013**	0.009**	0.011**	0.012**	0.0013**
Zn	0.0078*	0.0016**	0.0078**	0.0016*	0.009**	0.011**	0.014**	0.0016**
MxF	0.0090**	0.0019*	0.0090*	0.0019*	NS	0.017*	0.015*	0.0019*
MxZ	0.011*	0.0023*	0.011*	0.0023*	NS	0.017*	NS	0.0023*

\*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , NS not significant (Balakrishnan 2011), Fe<sub>1</sub>-FeSO<sub>4</sub> @ 12.5 kg ha<sup>-1</sup>, Fe<sub>2</sub>-FeSO<sub>4</sub> @ 25 kg ha<sup>-1</sup> and Zn<sub>1</sub>-ZnSO<sub>4</sub> @ 12.5 kg ha<sup>-1</sup>, Zn<sub>2</sub>-ZnSO<sub>4</sub> @ 25 kg ha<sup>-1</sup>.s

reports that mycorrhizal fungi can increase soil enzyme activities, such as phosphatase (Kothari et al., 1991; Mar Vazquez et al., 2000), dehydrogenase, urease, protease and  $\beta$ -glucosidase (Caravaca et al., 2003, 2004). The mechanisms on the enhancement of soil enzymatic activities may involve direct and indirect roles of mycorrhizal fungi: (1) mycorrhizal propagules themselves synthesize soil enzymes. It is reported that mycorrhizal fungal hyphae can produce some hydrolytic enzymes (Varma, 1998); (2) mycorrhizal plants may release more root exudates containing soil enzymes than that of non-mycorrhizal plants because of the larger root system and/or improved nutrition and/or resistances to stress of mycorrhizal plants.

Mar Vázquez et al. (2000) reported mycorrhizal colonization induced qualitative changes in the

microbial population and enzyme activities in the rhizosphere of maize plants. On the other hand, soil phosphatase and urease are closely related to the P and N nutrition of plants. Thus, the enhancement of soil enzyme activities is one of the physiological and biochemical mechanisms involved in a mycorrhization effect on plant mineral nutrition. Rao and Tak (2001) reported mycorrhizal fungal inoculation resulted in enhanced plant growth, total uptake of N, P and many other nutrients, activities of dehydrogenase, phosphatases and nitrogenase in the rhizosphere in gypsum mine spoil; (3) mycorrhizal fungi alter soil microbial communities in the rhizosphere directly or indirectly through changes in root exudation patterns or through fungal exudates, the so called "mycorrhizosphere effect" (Linderman, 1992). Dehydrogenase activity

recorded 0.041 and 0.078 OD at 485 nm in calcareous and non-calcareous soil, respectively and phosphatase activity was 0.895 and 1.023  $\mu\text{g pNP released g}^{-1} \text{ min}^{-1}$  in calcareous and non-calcareous (Tables 3 and 4) soil, respectively (Balakrishnan, 2011).

**MYCORRHIZAS ON GLOMALIN CONTENT IN SOIL**

Glomalin is a glycoprotein produced by mycorrhizal fungi. Glomalin can be extracted from the hyphae of all the isolates of AMF examined to date (Rillig and Steinberg, 2002), and glomalin was secreted into soil through turnover of AMF. Glomalin is insoluble in water, but can be solubilized by 20 or 50 mmol L<sup>-1</sup> citrate at 121°C

**Table 4.** Influence of mycorrhizal inoculation on acid phosphatase ( $\mu\text{g}$  of PNP/g/min) at 45 and 75 DAS under Fe-Zn interactions (Mean of 4 replications).

Treatments	Calcareous								Non-calcareous							
	Sterilized soil				Natural soil				Sterilized soil				Natural soil			
	45 DAS		75 DAS		45 DAS		75 DAS		45 DAS		75 DAS		45 DAS		75 DAS	
	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>
Fe <sub>1</sub> Zn <sub>1</sub>	1.16	1.30	1.69	2.12	1.15	1.89	2.17	2.89	1.26	1.47	1.49	1.69	1.50	2.02	1.96	2.32
Fe <sub>2</sub> Zn <sub>1</sub>	1.18	1.28	2.13	2.29	1.27	1.82	2.20	3.21	1.28	1.55	1.62	1.90	1.56	1.96	2.26	2.39
Fe <sub>1</sub> Zn <sub>2</sub>	1.25	2.13	2.06	3.20	1.16	1.89	2.28	2.92	1.29	1.48	1.69	2.12	1.62	2.19	2.06	2.65
Fe <sub>2</sub> Zn <sub>2</sub>	1.17	2.15	2.19	3.43	1.30	1.96	2.13	3.12	1.30	1.87	1.86	2.29	1.56	2.24	2.45	2.56
Mean	1.20	1.70	1.95	2.76	1.22	1.89	2.20	3.03	1.28	1.59	1.67	2.00	1.56	2.10	2.18	2.48

**ANOVA: M (Mycorrhizal inoculation), F (Fe levels), Z (Zn levels)**

M	0.0020**	0.0030**	0.0017**	0.0040**	0.0027**	0.0063**	0.0036**	0.0021**
F	0.0020**	0.0030**	0.0017**	0.0040**	0.0027**	0.0063**	0.0050**	0.0021**
Zn	0.0025**	0.0035**	0.0021**	0.0045**	0.0029**	0.00603**	0.0061**	0.0025*
MxF	0.0029*	0.0038**	0.0024**	0.0048**	NS	NS	0.0070*	0.0028*
MxZ	0.0035**	0.0045*	0.0030*	0.0045*	NS	0.0082*	NS	0.0038*

\*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , NS not significant (Balakrishnan 2011), Fe<sub>1</sub>-FeSO<sub>4</sub> @ 12.5 kg ha<sup>-1</sup>, Fe<sub>2</sub>-FeSO<sub>4</sub> @ 25 kg ha<sup>-1</sup> and Zn<sub>1</sub>-ZnSO<sub>4</sub> @ 12.5 kg ha<sup>-1</sup>, Zn<sub>2</sub>-ZnSO<sub>4</sub> @ 25 kg ha<sup>-1</sup>.

(Wright et al., 1996); highly correlated with soil C and N (Rillig et al., 2001); sequesters potentially toxic elements in soil (Gonzalez-Chavez et al., 2004); linked with an oligosaccharide (Wright et al., 1996); strongly positively correlated with soil aggregate stability and has a relatively long lifespan in soil. Moreover, the effect of glomalin on soil aggregation is stronger than that of AMF (Driver et al., 2005) because glomalin acts as insoluble glue for the stabilization of aggregates (Wright et al., 1996).

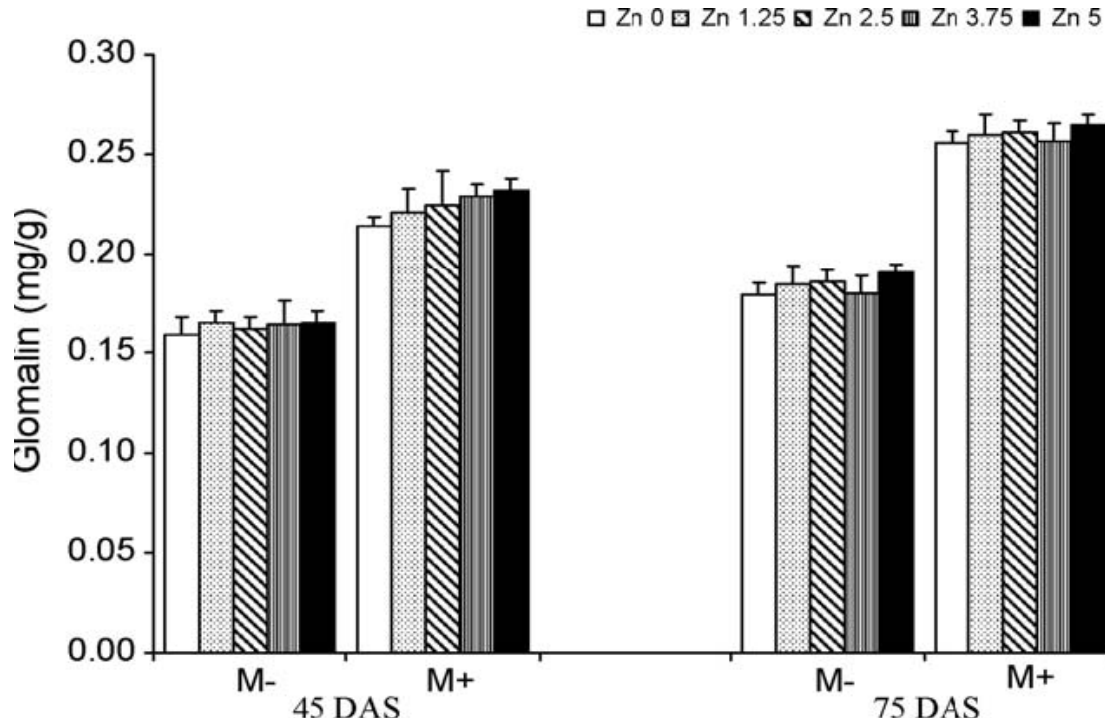
Furthermore, there is abundant glomalin in natural and cultivated soil, and the concentration of glomalin in soil ranges from 4.4 to 14.8 mg g<sup>-1</sup> soil (Wright et al., 1996). Lovelock et al. (2004) calculated that approximately 3.2% of total soil C and 5% of soil N in rain forest soil was in the form of glomalin, and according to Rillig et al. (2001) up

to 5% of soil C and 4% of soil N stocks were derived from glomalin. Miller and Jastrow (2000) estimated that hyphae and glomalin together contributed up to 15% of soil organic C in a grassland. In a tropical forest soil, glomalin carbon represents 4 to 5% of total soil carbon, much higher than soil microbial biomass carbon. The close correlation of the amount of glomalin in soil with hyphal length and the stability of soil aggregates suggested that glomalin could influence soil carbon storage indirectly by stabilizing soil aggregates. Subramanian et al. (2009) indicated that mycorrhizal symbiosis increased the glomalin protein concentration in arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenck and Smith) inoculated (+Myc) soil by 40% in comparison to uninoculated (-Myc) soil (Figure 3).

## MYCORRHIZAS ON BIOFORTIFICATION

Biofortification is a process in which plants are allowed to take up the minerals (Fe and Zn) from the soil and immobilize them in the grains so as to produce nutritionally rich grains that support dietary requirement of humans. This approach has proved to be sustainable, relatively low cost, highly efficacious and large coverage (Poletti et al., 2004). Biofortification requires that agricultural research makes direct linkages with the human health and nutrition sectors (Bouis, 2003). This requires a multidisciplinary research approach, a willingness among scientists to communicate across disciplinary boundaries, and innovative funding strategies to support the research and ultimate dissemination of the biofortified seeds.

The mycorrhizal symbiosis assists in



**Figure 3.** Glomalin concentrations in mycorrhizal inoculated (+Myc) and uninoculated (-Myc) soil at 45 and 75 DAS under different levels of Zn. The datasets at 45 and 75 DAS were analyzed separately. Error bars represent standard errors of three replications (Subramanian et al., 2009).

biofortification of micronutrients such as Fe and Zn in maize grain irrespective of calcareous and non-calcareous soils. The mechanism involved in improved micro nutrition of maize includes acidification of rhizosphere; siderophore production enhanced physiologically active Fe and production of anti-oxidants (Table 5) besides synergistic interaction between P and micronutrients (Balakrishnan, 2011).

### MYCORRHIZAS ON DROUGHT RESISTANCE

The inoculation with *Glomus intraradices* improves drought tolerance of tomato plants as secondary consequence of enhanced nutritional status of the host plant, especially N and P. Mycorrhizal association improved tomato fruit quality by enhancing ascorbic acid content and reducing the acidity (Subramanian et al., 2006). Mycorrhizal colonization by *G. intraradices* improved nutritional status and N assimilation in maize plants exposed to moderate drought stress. The increased capacity for N acquisition and assimilation may enable the host plant to sustain moderate drought stress conditions. Glutamine synthetase activity in AM plants increased by 30% under drought conditions, which may be attributed to the hyphal transport of N in the form of  $\text{NO}_3$  or  $\text{NH}_4$  (Subramanian and Charest, 1999). They

concluded that mycorrhizal colonization may improve the water status of non-irrigated vines (Nikolaou et al., 2003).

### INTERACTION OF AM AND AGRICULTURAL PRACTICES

Agricultural practices such as fertilizer applications, crop rotation, tillage, and liming affect field AM potential and root colonization levels. Higher soil infectivity was observed under reduced or no tillage practices (Mozafar et al., 2000) and liming increased mycorrhizal colonization of barley roots and soil infectivity (Hamel et al., 1996). Under field conditions, beans, corn, and leek have a much higher mycorrhizal dependency than potato and wheat. This range of plant response to AMF has to be taken into account when managing a cropping system or a crop rotation. Data on the potential of crop plants to benefit from mycorrhizal symbiosis are available at the mycorrhizal producers level clearly explained and the investigations done by Plenchette et al. (1983) gives examples of relative field mycorrhizal dependency (RFMD) for some plants. Equation 1 gives the formula for calculating RFMD (Table 6).

$$\text{RFMD} = \frac{\text{DM of mycorrhizal plant} - \text{DM of non-mycorrhizal plant}}{\text{DM of mycorrhizal plant}} \times 100$$



**Table 5.** Influence of mycorrhizal colonization on grain nutrient content (Fe and Zn (mg kg<sup>-1</sup>)) of maize under Fe- Zn interactions (Mean of 4 replications).

Treatments	Iron								Zinc							
	Calcareous				Non-calcareous				Calcareous				Non-calcareous			
	Sterilized soil		Natural soil		Sterilized soil		Natural soil		Sterilized soil		Natural soil		Sterilized soil		Natural soil	
	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>	M <sup>-</sup>	M <sup>+</sup>
Fe <sub>1</sub> Zn <sub>1</sub>	23.6	28.7	32.1	44.1	26.1	30.9	42.5	48.7	17.3	24.9	23.5	37.5	18.7	28.2	30.5	40.6
Fe <sub>2</sub> Zn <sub>1</sub>	24.7	30.1	39	53.7	31	36.8	50.5	57.9	19.7	24.5	26.8	42.8	21.3	27.3	34.7	46.2
Fe <sub>1</sub> Zn <sub>2</sub>	26.5	32.1	36	49.5	27.8	32.9	45.3	51.9	18.7	26.3	25.4	40.5	20.1	29.3	32.8	43.6
Fe <sub>2</sub> Zn <sub>2</sub>	28.7	33.6	43.5	59.9	34	40.4	55.4	63.4	20.9	28.6	28.4	45.4	22.6	28.6	36.8	48.9
Mean	25.9	31.1	37.7	51.8	29.7	35.3	48.4	55.5	19.2	26.1	26.0	41.6	20.7	28.4	33.7	44.8

**ANOVA: M (Mycorrhizal inoculation), F (Fe levels), Z (Zn levels)**

M	0.051**	1.35**	0.92**	1.10**	0.030**	1.38**	0.59**	1.84**
F	0.049*	1.97**	0.26**	1.66**	0.076**	1.13**	0.05*	1.65**
Zn	NS	1.21**	NS	1.62**	NS	1.78*	NS	1.25*
MxF	0.087*	NS	0.64*	2.53*	NS	2.54*	NS	2.00*
MxZ	NS	NS	NS	NS	0.68*	2.26*	0.11*	2.04*

\* P ≤ 0.05, \*\* P ≤ 0.01, NS not significant (Balakrishnan 2011), Fe<sub>1</sub>-FeSO<sub>4</sub> @ 12.5 kg ha<sup>-1</sup>, Fe<sub>2</sub>-FeSO<sub>4</sub> @ 25 kg ha<sup>-1</sup> and Zn<sub>1</sub>-ZnSO<sub>4</sub> @ 12.5 kg ha<sup>-1</sup>, Zn<sub>2</sub>-ZnSO<sub>4</sub> @ 25 kg ha<sup>-1</sup>.

**STRATEGIES TO PROMOTE MYCORRHIZAL SYMBIOSIS****Minimal soil disturbance**

Heavy tillage has a major effect on the symbiosis, reducing root colonization primarily by disrupting the hyphal network (Evans and Miller, 1988). No till system favours mycorrhizal symbiosis and therefore their adoption would enhance the functions of mycorrhiza and aid the sustainability of the system.

**Fertilizers**

The literature on fertilizer impact on colonization of mycorrhiza is contradictory. Application of soluble P appears to reduce the germination of

mycorrhizal spores and extent of colonization (Miranda and Harris, 1994). However, some fungal species (*Glomus intraradices*) are insensitive to fertilizer application.

**Crop rotations**

Mycorrhizas are obligate symbionts and thus highly dependent on living host plants. Most agricultural crops such as flax, corn, sorghum, wheat, barley, potatoes, and sunflower can benefit from mycorrhizal association (Dalpé and Monreal, 2004). Fallowing also results in reduction of inoculum potential but the effect is smaller than the inclusion of non-mycotrophic plants in the cropping sequence (Wu et al., 2004; Panja and Chaudhuri, 2004).

**Pesticides**

Mycorrhizas are highly sensitive to pesticides such as methyl bromide and if used, it impedes the development of mycorrhizal symbiosis in the crop. Most fungicides have shown to be detrimental to either colonization or functions of mycorrhizas. Rationalization of their use by selection of fungicides that do not interact negatively with mycorrhiza could bring significant benefits.

**Crop breeding**

Some crop varieties are more responsive to mycorrhizal colonization and can be exploited for low-input production system (Subramanian and Charest, 1997).

**Table 6.** Relative field mycorrhizal dependency (RFMD) for selected plants.

Plant name	RFMD* (%)
Cabbage (Brassicaceae)*	0
Carrot	99.2
Chicory (witloof)	82.4
Faba bean	93.5
Garden beet (Chenopodiaceae)*	0
Garden pea	96.7
Kentucky blue grass	72.4
Kidney bean	94.7
Leek	95.7
Pepper	66.1
Potato	41.9
Tomato (according cultivars)	59.2 - 78.0
Sweet corn	72.7
Wheat (according cultivars)	44.5 - 56.8

\*Non-mycorrhizal plant.

## TECHNIQUES FOR EXTRACTION ARBUSCULAR MYCORRHIZAL FUNGI

This will provides an overview on present techniques used to extract AM fungi in soil and roots.

### Spore extraction

Several techniques for the extraction of AM fungi spores from soil have been reviewed by Hayman (1984) and Schenck and Perez (Schenck and Perez, 1990). The wet sieving and decanting as described by Gerdeman and Nicolson (1963), followed by sucrose density gradient centrifugation technique as described by Daniels and Skipper (1982), Schenck and Perez (1990) is the most widely used technique. The major variable in its application is in the use of single or multiple densities of sucrose (Jarstfer and Sylvania, 2002). Multiple layers of different densities can provide cleaner spores and are useful for separating different species. Another method of separating spores from debris uses a series of sieves of various pore sizes. As with most of the techniques, it works best for sandy soils and less well for clay or organic soils (Jarstfer and Sylvania, 2002). Soil samples with significant clay content can be soaked in 6.3 mMol of sodium hexametaphosphate to disperse the clay fraction (Miller et al., 1995).

### Wet sieving and decanting technique

After collection of a soil samples, suspend approximately 50 to 100 g of soil into a 2-liter container and add 1.5 L of water. Vigorous mix the suspension to free the spores

from the soil and roots. For fungal species that form spores in roots (e.g. *Glomus intraradices* and *Glomus clarum*), blend the soil-root sample for 1 min in 300 ml of water to free the spores from roots. Next, heavier particles in suspension is allowed to settle for 15 to 45 s (times vary depending on soil texture) and the supernatant decanted through standard sieves. Sieves should be selected so as to capture the spores of interest. A 425 µm pore size over a 45 µm pore size sieves is used for unknown field samples. The content of the top sieve is examined for Sporocarps that may be up to 1 mm in diameter. For clay soils, it is advisable to repeat the decanting and sieving procedure with the settled soil. Roots may be collected from the larger mesh sieve for evaluation of internal colonization. The sievings retained on the other different sieves were washed into separate Petri dishes for further observations or purification by sucrose centrifugation (Gerdemann and Nicolson, 1963).

### Summary

The key effects of Arbuscular Mycorrhiza (AM) can be summarized as follows: (i) improved rooting and plant establishment; (ii) improved uptake of Fe and Zn; (iii) improved nutrient cycling; (iv) enhanced plant tolerance to biotic and abiotic stress; (v) improved crop yield; (vi) quality of soil structure; (vi) enhanced plant community diversity; (vii) reduces water usage; (viii) reduce fertilizer usage (approximately 15 to 25%); (ix) improved below and above ground carbon sequestration. Hence, AM is a bio bridge between plant and soil which ensure greening in dry land and the use of its as a bio fertilizer can be regarded as an important alternative strategy for sustainable agriculture.

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