ARBUSCULAR MYCORRHIZAL FUNGI (AMF) A KEY COMPONENT OF THE SYMBIOTIC SOIL MICROBIAL POPULATIONS - A CRITICAL STUDY

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Abstract -Arbuscular mycorrhizal fungi (AMF) are one of the most important microbial symbionts in the roots of maximum plants on the Earth. Commonly, AMF are non host specific and have symbiotic interactions with different plants species. But some AMF species at certain combinations with host's plant species have shown some host specificity. The beneficial interactions of AMF with plants growth result in the improvement of plant health and soil fertility. Under stress conditions of plants and especially in phosphate-limitated condition, AMF can influence plant community development, nutrient uptake, water relations and aboveground productivity. They can also act as bioprotectants against pathogens and toxic stresses.AMF have different types of interactions with different kinds of rhizosphere microorganisms, particularly with rhizospheric bacteria that exhibiting some specific characteristics and functions in associations of them.

These interactions may result in spore formation and germination through root colonization to external hyphae during AMF life cycle. The nature of these bacteria-fungal interactions may be inhibitory or stimulatory, competitive or mutualistic to each other or for the plant. In nature, the plant species impact on the selection of their bacterial associates and strongly influence the composition of AMF community. Indirectly AMF species also influence on the selection of bacteria in the mycorrhizosphere. Several kinds of bacterial species have been studied that are present in plant-AMF mycorhizosphere, but bacteria associated with AMF spores (AMB) with different interaction are not well studied yet. These AMB may be involved in the biocontrol of plant pathogens and to improve supply of nutrients. The knowledge of interactions between plant AMF, AMB and plant pathogens have been found helpful for the development of sustainable management of soil fertility and to increase crop production.

I INTRODUCTION

Frank in 1885 for the first time coined the term mycorrhiza (mykes =fungus, rhiza= root) to describe the symbiosis between a soil fungus and plant roots. These soil fungi make various types of associations with plant roots. Different types of mycorrhizal associations based on the type of fungus involved and the resulting structures produced by the root–fungus combination have been identified; *e.g.* AMF(Arbuscular Mycorrhizal Fungi) ectomycorrhiza, ectendomycorrhiza, ericoid, arbutoid, orchid and monotropoid. The AMF symbiosis is generally mutualistic in which bi-directional nutrient transfers between the host plant and the fungus takes place and where carbon flows to the fungal partner and different nutrients move to the plant (Smith & Read, 1997).

It has been estimated that AMF are the most common mycorrhiza that colonize about 80 % of plant families from all terrestrial plants (Schüßler, Schwarzott & Walker, 2001). AMF are strictly obligate i.e. AM fungi need the living plant roots to survive. But some reports showed that AM species can grow upto the spore production phase *in vitro* in the absence of plant roots and in the presence of some selected strains of sporeassociated bacteria (Hildebrandt, Janetta & Bothe, 2002; Hildebrandt *et al*., 2006). The AM fungal root colonization is affected by the type of plant species *e.g.* host *vs* non-host plant, presence of inoculum level in the rhizosphere, AM fungal species and its associated microbes, phosphorus availability of soil (Hayman, 1982), soil water content (Jasper, Abbott & Robson, 1993), temperature (Kobayashi, 1988), soil pH, and agrochemical applications.

II CLASSIFICATION, IDENTIFICATION AND DIVERSITY

AMF are recognized based on their specific traits such as obligate biotrophy, asexual reproduction, large and multinucleate spores with layered walls, non-septate hyphae and arbuscules formation in plant roots. AMF reproduce asexually by spore production. There is no evidence that AMF reproduce sexually (Kuhn *et al*., 2001). Only low level or no genetic recombination has been detected using molecular marker genes (Kuhn *et al*., 2001). Therefore, it is generally assumed that the AMF spores are formed asexually. The spores are relatively large (40-800 µm) with layered walls and lipids in their cytoplasm. Spores are important for identification of AMF. Traditionally AM fungal taxonomy has been based on the spore morphology particularly spore wall layer structure and the way of spore formation on the hypha (Morton, 1998). AMF under Phyllum Glomeromycota consists of four orders, nine families and twelve genera. AMF are found to be the sister group of *Ascomycota* and *Basidiomycota* but not the monophyletic with any part of the *Zygomycota* based on rDNA phylogeny. In earlier classification AMF were placed in order Glomales within the division *Zygomycota* because AMF have non- septate hyphae, a similar characteristic to that found in most of the *Zygomycota*. However, AMF are distinguished from the Zygomycotan lineages due to some specific characteristics *e.g.* mutualistic symbiotic nutritional habit and lack of formation of characteristic zygospores. The rDNA analysis exposed a clear separation of AMF from other fungal groups (Schüßler, Schwarzott & Walker, 2001) and AMF are now placed in a separate new phylum *Glomeromycota* (Schüßler, Schwarzott & Walker, 2001). The phylum *Glomeromycota* is divided into four orders, eight families and ten genera (Walker & Schüßler, 2004) and it consists of more than 150 species that have been identified on the basis of spore morphology (Redecker & Raab, 2006).

Based on molecular studies 200 species have been identified. These studies indicate a strong estimation of true diversity of Glomeromycotan fungi that have been described as morphospecies (Husband *et al*., 2002; Vandenkoornhuyse *et al*., 2002). Recently, two new AMF genera, *Kuklospora* and *Intraspora* have been incleded in the phylum *Glomeromycota* (Sieverding & Oehl, 2006).

Classification of AMF under Phylum *Glomeromycota* with different orders, families and genera are described in the present figure 1

PHYLUM GLOMEROMYCOTA CLASS GLOMEROMYCETES		
Orders	**Families**	**Gendera**
Glomerales	Glomeraceae	Glomus
Diversisporales	Gigasporaceae	Gigaspora, Scutellospora
	Acaulosporaceae	Acaulospora, Kuklospora
	Enterophosporacea	Enterphospora
	Pacisporaceae	Pacispora
	Diversisporaceae	Diversispora
Paraglomerales	Paraglomeracea	Paraglomous
Archeosporales	Geosiphonaceae	Geosiphon
	Archaeosporaceae	Archaeospora, Intraspora

Table 1: Recent classification of arbuscular mycorrhizal fungi (Sieverding & Oehl, 2006)

Eom *et al*., (2000) have described that plant species play important role in the regulation of species composition and diversity of AM fungal communities. AMF exhibits a great diversity in rhizosphere in which different types of interactions occur between soil microbes and plants. These interactions can influence plant community development, nutrient uptake, water relations and aboveground productivity. The differences observed in AMF diversity depends on the type of ecosystems, agricutural practices, soil conditions and methods used for AMF identification.

AMF are identified on the basis of classical spore morphology produced by them. Nowadays by using modern techniques of PCR-based molecular approaches are generally used for identification of AMF. Both of these approaches are not well satisfied and have problems. By using spore techniques, it is not always possible to identify all the spores. Spore may have different morphological structures produced during their different developing stages. Sometimes their morphological structures may be distorted or disturbed during sieving from soil and or during isolation and

separation of the spores by using different techniques of isolations. On the other hand AMF in plant roots donot produce spores and sometimes only mycelium are present colonizing the roots (Clapp *et al*., 1995; Clapp, Rodriguez & Dodd, 2002).

By using molecular approaches, the main problem is that most approaches are based on rDNA sequences. AMF species have polymorphic rDNA sequences (Sanders, 2002; Redecker, Hijri & Wiemken, 2003). Thus, it is always normal to recover multiple sequences by PCR amplifications from a single spore as a single spore can contain a thousand or more nuclei (Antoniolli *et al*., 2000; Pawlowska & Taylor, 2004). Presently, there are no individual rDNA primers that permit identification of all major Glomalean lineages and most molecular approaches used till date are not able to detect all rDNA sequences present (Redecker, 2000; Vandenkoornhuyse *et al*., 2002; Redecker, Hijri & Wiemken, 2003; Schüßler, Schwarzott & Walker, 2003). Thus the identification of AMF communities based on either spore morphology (Landis, Gargas & Givnish, 2004) or molecular identification alone is insufficient to cover the whole spectrum within a community. Therefore, both of these approaches are recommended as they complement each other (van der Heijden & Scheublin, 2007).

III LIFE CYCLE

The life cycle of mycorrhiza starts from the spores present in soil or from adjacent plant roots harboring mycorrhizal mycelia. Hypha(H) emerge from spores or mycorrhizal roots grow towards the adjacent plant root. On the surface of the root the tip of hyphae swells at the point of attachment and forms a specific structure called appressorium (Ap) (Mandelbaum & Piche, 2000). Then from appressoria, the infective pegs (Ip) emerge to enter inside host root. Hyphae penetrate the adjacent epidermal root cell walls with the help of penetration pegs. This particular point from where for the first time hyphae from any propagule enters the root is called a primary entry point (E) (Fig 1). The number of primary entry points formed on a root surface by a fungus is equivalent to its inoculum potential (Garrett, 1956; Bouhot, 1979). After the entry of hyphae inside the root, hyphae grows intercellularly. Inside the cells, the host cell membrane invaginates and envelopes the fungus. The hyphae form different special types of structures such as hyphal coils, characteristic arbuscules (Ar) and vesicles (V) inside the cortical cells. These structures are formed outside the cytoplasm. Arbuscules are highly dichotomously branched intracellular structures and could be the site of exchange for phosphorus, carbon, water and other nutrients (Wright, 2005; Smith & Read, 1997). Vesicles are lipid-filled structures of special characteristics and thought to be carbon storage structures but they can also serve as reproductive propagules (Sylvia, 2002). It is not necessary that all AMF form vesicles. Formation of the vesicles depends on the fungal symbiont as well as on the environmental conditions (Smith & Read, 1997).

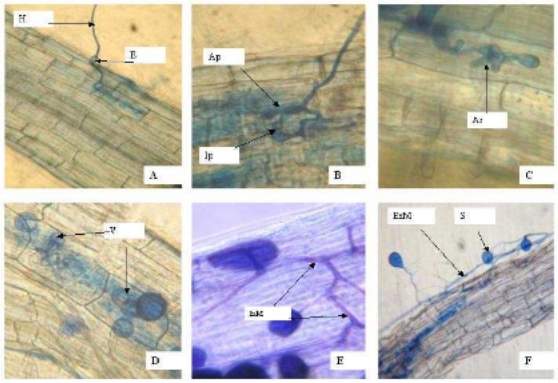

Figure 1: Different structures involved in AMF life cycle were observed in potato roots during this study: hyphae (H), entry point (E), appresorium (Ap), infection peg (Ip), arbuscule (Ar), vesicle (V), intraradical mycelium (InM), extraradical mycelium (ExM) and spore (S).

Once the infection has begun, colonization starts both within a root by intraradical mycelium (InM) and along the root by the extraradical mycelium (ExM).The intraradical mycelium inside the root grows in different patterns. Mycorrhiza have been categorized in three different groups based on the structure of colonized intraradical mycelium as *Arum*, *Paris* and *Intermediate* type (Gallaud, 1905). In *Arum*-type, intercellular hyphae grow in a longitudinal manner through the root and penetrate the root cortical cells to form arbuscules. Arbuscules arise on short side branches from these intercellular hyphae typically at right angles to the main root axis (Smith & Smith, 1997). The *Arum*-type morphology is abundant in crop plants (Smith & Smith, 1997; Ahulu, Nakata & Nonaka, 2005). In *Paris*-type, intercellular hyphae are absent and the hyphae are entirely intracellular with irregular coiled hyphae, some of which form arbuscules that are not terminal but are

localised in definite layers. The arbuscules are formed as intercalary structures and called as arbusculate coils (Gallaud, 1905; Yawney & Schultz, 1990; Cavagnaro *et al*., 2001). *Paris*-type morphology is found in plants of natural ecosystems (Brundrett & Kendrick, 1988; Ahulu, Nakata & Nonaka, 2005). Sometimes, both types are present in the same root system than it is called the *Intermediate*-type (Smith & Smith, 1997) found in plants cucumber and tomato (Kubota, McGonigle & Hyakumachi 2005).

The extraradical mycelium is found attached with the roots that radiates out into the soil. Two different types of hypha are produced by extraradical mycelium termed as runner and absorbing (Friese & Allen, 1991). The runner hypha are thicker, grow in the soil and penetrates the host roots. The absorbing hypha always develop from the running hypha and form a network of thinner hyphae extending into the soil. Their main function is to absorb the nutrients from the soil and supply to the host plant. Some mycorrhizal species produce typical clustered swellings that are formed on extraradical hyphae called auxiliary cells *e.g. Gigaspora* and *Scutellospora* species. The function of these structures is yet to be known.

Reproductive structures of mycorrhiza are spores (S) be formed as hyphal swellings. Hypha swell up either in the roots or, more commonly, in the soil to produce spores .Spores may be formed singly or in clusters. Spores are thought to be mainly as storage structures, resting stage and propagules (Brundrett *et al*., 1996).

IV AMF INTERACTION WITH PLANTS

About 80% of land plants have been found associating mycorrhiza. AMF colonize the host roots by forming intercellular and intracellular hyphae and intracellular arbuscules. Plants species belonging to family Cruciferae and Chenopodiaceae are known to be either non-mycorrhizal or non hosts of AMF (Smith & Read, 1997).

AMF are non host specific or have very low host specificity. Most of the AMF colonize a wide range of hosts and the same plant root can be colonized by a mixture of AMF species. Some studies indicate that plants might select AM fungus (van der Heijden *et al*., 1998) and Vandenkoornhuyse *et al*., (2002) observed that different AMF type were found associated with different plants.

V AMF AND SOIL BACTERIA

The mycorrhizosphere is the soil surrounding and influenced by the mycorrhizal fungi (Rambelli, 1973), where the fungus colonizes the roots and modifies the root soil aggregation and water distribution in the soil through its extramatrical hyphae (Andrade *et al*., 1998). In mycorhizosphere AMF interact with different types of microorganisms present in soil. Particularly soil bacteria influence the development and symbiotic establishment of AMF. The interactions between AMF and bacteria can be positive (Bagyaraj & Menge, 1978) or negative (Gryndler, Hrselova, & Chvatalova, 1996; McAllister *et al*., 1995) or neutral (Edwards, Young & Fitter, 1998). In positive interactions development and function of mycorrhiza is enhanced. Synergistic positive interactions have been reported as nitrogen fixers, fluorescent pseudomonads and sporulating bacilli (Hameeda *et al*., 2007). During negative interactions it can reduce spore germination, hyphal length, root colonization and metabolic activity of the mycelium.

It is believed that the AM symbiosis reduces phosphate stress resulting in enhanced N_2 fixation that indirectly promotes plant growth and mycorrhizal development (Bethlenfalvay, 1992; Fraga-Beddiar & Le Tacon, 1990).

These interactions lead to changes in the microbial composition (Hodge, 2000). The change in bacterial populations can take place due to several modes; *e.g* competition for nutrients, changes in soil structure, changes in plant root exudate patterns and energy- rich compounds provided by the extra-radical mycelium of AM fungi (Andrade *et al*., 1997; Tisdall & Oades, 1979; Ravnskov, Nybroe & Jakobsen, 1999; Söderberg, Olsson & Bååth, 2002; Mayo, Davis & Motta, 1986). The composition of bacterial populations in mycorrhizosphere of AM plants can affect the interaction between plant and AM fungi (Andrade *et al*., 1997). There are reports that indicate some bacteria have been found associated with AMF mycelium e.g. Genus *Paenibacillus* intimately associated with the mycelium of *G. intraradices* (Mansfeld-Giese, Larsen

& Bodker, 2002) and *Bacillus cereus* with *G. dussii* (Artursson & Jansson, 2003). It reveals that some bacteria are more specific to particular type of AMF, which might be due to the secretion of specific exudates by specific AMF species (Artursson & Jansson, 2003). Mosse, (1962) for the first time reported that bacteria colonize the spores of AM fungi. Different studies have since then shown that the spore-associated bacteria can influence the germination of AMF spores, the growth of AMF (Bianciotto & Bonfante, 2002; Hildebrandt, Janetta & Bothe, 2002; Walley & Germida, 1996; Xavier & Germida, 2003) and on the formation of the mycorrhizosphere (Budi *et al*., 1999). Mechanisms controlling associations of bacteria with AMF and plant roots in the mycorrhizosphere (Artursson, Finlay & Jansson, 2005, Bharadwaj *et al*., 2008) are not fully elucidated. But a deeper understanding about interactions between the AM fungi and their associated bacteria can partly be gained by characterizing the bacterial spectrum of different habitats in the plant rhizosphere.

VI AMF IN DISEASE CONTROL

Meyer & Linderman, (1986) reported for the first time the role of the mycorrhizosphere in biocontrol of pathogens. They found that the extracts of rhizosphere soil from mycorrhizal plants reduced sporangia formation of *Phytophthora cinnamomi* in comparison with extracts of rhizosphere soil from non-mycorrhizal plants. These authors hypothesized that either the sporulation-inducing microorganisms were missing or that the number of sporulation-inhibiting microorganisms increased.

Reports are available indicating that AM fungi play an important function in the reduction of plant pathogens (St-Arnaud *et al*., 1995; Azcón-Aguilar & Barea, 1996; Whipps, 2004) such as *Fusarium oxysporum* (Dehne & Schönbeck, 1979; Caron, Richard & Fortin, 1986; St-Arnaud *et al*., 1997; Filion, St-Arnaud & Fortin, 1999), different *Phytophthora* species (Davis & Menge, 1980; Cordier, Gianinazzi & Gianinazzi-Pearson, 1996; Trotta *et al*., 1996), *Rhizoctonia solani* (Yao, Tweddell & Desilets, 2002) and *Pythium ultimum* (Calvet, Pera & Barea, 1993) in different crops. AMF has also been shown to reduce the bacterial diseases (Dehne, 1982) and affect the nematode development (Diedhiou *et al*., 2003; Ryan *et al*., 2000; Talavera, Itou & Mizukubo, 2001). *G. intraradices* is shown to suppress the *Fusarium sambucinum,* causal organism of potato dry rot (Niemira, Hammerschmidt, & Safir, 1996) and *R. solani* (Yao, Tweddell & Desilets, 2002) and *G. etunictum* suppress the *R.solani* in potato (Yao, Tweddell & Desilets, 2002). The mode of action of biocontrol activity of AM fungi is assumed to be the direct interactions between AM fungi and pathogens but mycorrhiza-mediated triggering of plant defense reactions have also been proposed (Azcón-Aguilar & Barea, 1996; Whipps, 2004). In addition, antagonism from bacteria inhibiting mycorrhizosphere has also been suggested as a possible mechanism (Budi *et al*., 1999).

VII AMF IN DISEASE CONTROL

Secilia & Bagyaraj, (1987) also found that the antagonistic actinomycetes got increased in the rhizosphere of mycorrhizal plants compared to that in the rhizosphere of non-mycorrhizal plants. Budi *et al*., (1999) reported that a *Paenibacillus* strain isolated from surface-sterilized *G. mosseae* spores inhibited a number of different plant pathogens *viz. Aphanomyces euteiches*, *Chalara elegans*, *Pythium* sp., *Fusarium culmorum*, *F. oxysporum*, *Phytophthora parasitica* and *R. solani.* There are some studies on bacteria associated with AMF spores such as *Pseudomonas*, *Bacillus* (Meyer & Linderman, 1986), *Burkholderia* (Mao *et al*., 1998), *Paenibacillus* (Budi *et al*., 1999) and *Streptomyces* (Secilia & Bagyaraj, 1987) showing that AM have the potential to control plant pathogens.

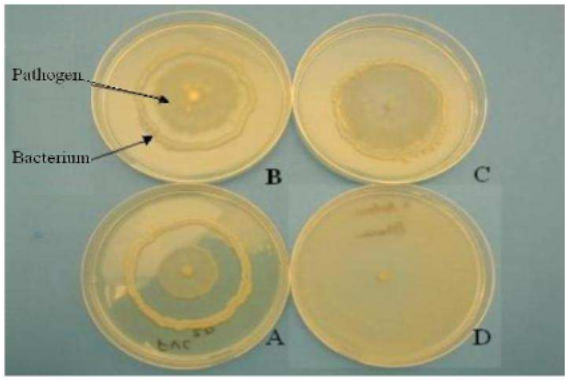

Figure 3: Effect of three different AMB isolates inhibiting the radial growth of *Rhizoctonia solani*. A=Strong inhibition, B= Moderate inhibition, C= Weak or no inhibition, and D= control (*R. solani* only) (Bharadwaj, Lundquist & Alström 2012

Some AMB have antagonistic potential against several soil-borne pathogens *in vitro* and against *Phytophthora parasitica* also *in vivo* Budi *et al*., (1999). The antagonistic potential of spore-associated bacteria against pathogens needs to be fully explored in order to obtain information on the plant health promoting effect of the mycorrhizosphere. Interest in research on spore-associated bacteria has increased because these have shown potential to support fungal growth to complete spore production *in vitro* in the absence of a host (Hildebrandt, Janetta & Bothe, 2002). Plant pathogens sharing the same niche compete with plants and their associated microflora. The knowledge about the specific effects of individual plant species on composition of AMF and AMB and also the ecological function of these AMB in the development of AMF and plants is still very limited. Hence it has become a thrust area of study in plant-mycorrhiza associated bacteria.

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