

ECOLOGICAL SERVICES OF ARBUSCULAR MYCORRHIZAL FUNGI- A REAPPRAISAL

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(Received 29 August, 2020; accepted 13 October, 2020)

ABSTRACT

Arbuscular Mycorrhizal fungi (AMF) are ubiquitous and among the commonest symbiotic association between plants and microbes. It occurs in the majority of natural habitats because they provide a range of important ecological services by improving plant nutrients, pathogen resistance and stress tolerance, soil structure and fertility and many more. It also interacts with most crop plants including cereals, vegetables, and fruit trees. Basic research of the past decade has revealed the existence of a dedicated recognition and signaling pathway that is required for AM. Moreover, current facts provided novel insight into the trade of nutritional payback between the two symbiotic associates. Their importance in agricultural and forestry resides in their role in plant growth and nutrition. Dual inoculation of fungi with a *Rhizobium* and other bacterium on plant enhance the growth and other beneficial effects viz., resistance to disease and increase tolerance to adverse soil and climatic conditions. Hence, they are considered most promising agent with immense potential use in sustainable agriculture. This review highlights diverse role of Arbuscular mycorrhizal fungi in plant growth and development regulation, specifically under stress conditions.

KEYWORDS : AM fungi, Biocontrol, Plant defence, Bioremediation, Soil structure

INTRODUCTION

Arbuscular mycorrhizae, a Glomeromycota fungi, have existed since the appearance of the first plants occurred on dry land. They can be defined as a symbiotic relationship between fungi and plant roots. They are commonly known as mycorrhizae, taken from the Greek words “mukés”, meaning fungus, and “rhiza,” meaning roots (Panhwar *et al.*, 2009). These fungi form filamentous network with plant roots known as symbiotic mycorrhizal association, enhance root surface area, draw superfluous nutrients from the soil and supply them to plants. This mechanism stimulates plant growth and accelerates root physiology and development. Around one kilo meter of AM fungal hypae is associated with a plant growing in a one-litre pot and can access water and nutrients in the smallest

pores in the soil. It makes the plant less susceptible to soil-borne pathogens and to other environmental stresses such as drought and salinity. In return fungi gain carbohydrates and other nutrients from the plant. Fungi consume these carbohydrates for their growth and to synthesize and excrete specific molecules like Glomalin (glycoprotein). The release of Glomalin in the soil environment results in better soil structure and higher organic matter content (Çekic *et al.*, 2012).

Nevertheless, human activities results in the loss of mycorrhizal associations in the soil radically consequently significant decrease also occurs in the benefits imparted by mycorrhizae on plant growth and health. These symbiotic relationship function as a bridge for the flow of energy and matter between plants and soils (Sharma *et al.*, 2013). The symbiotic association involves most plant species and certain

fungal species which has great relevance to soil ecosystem functions, especially nutrient dynamics, microbial processes, plant ecology and agriculture.

TYPES OF MYCORRHIZAE

There are major two groups of mycorrhizal fungi: ectomycorrhizal and endomycorrhizal fungi. Among the types of endomycorrhizal fungi, arbuscular mycorrhizal (AM) fungi are most prevalent in 80% of plants in soils. Their name is derived from specific structures which they form within the plant root cell: Arbuscules. These are finely-branched structures that are formed within a cell and serve as a major metabolic exchange site between the plant and the fungus. Vesicles are also present in some species of AM fungi, which are sac-like structures, emerging from hyphae, and serve as storage organs for lipids. Rest types of mycorrhizae are highly specific to plant like ericaceous families and orchids also exist in nature. The fungi involved in the mycorrhizal colonization of these plant families are currently not available as commercial products. Ectomycorrhizal fungi mostly confined to forests ecosystems are also found in natural environments. They can form visible reproductive structures (mushrooms) at the feet of trees they colonize. Ectomycorrhizal fungi can grow between root cells without penetrating them. Their hyphae grow externally, forming dense growth known as a fungal mantle. These fungi form symbiotic relationships with most pines, spruces and some hardwood trees including beech, birch, oak and willow (Hakeem *et al.*, 2016).

According to plant species and to the growing practices and conditions, mycorrhizae provide different benefits to the plants and to the environment.

Prospective of Mycorrhizae as Biocontrol Agent

The biocontrol system in plants can be described as the management of frequent components to protect plants against pathogens. A number of findings showed that the mycorrhizal inoculation directly or indirectly increased plant defence against various types of pathogen. AMF inoculation improved plant nutrition; compensated the root damage and changed rhizospheric microbial populations and augmented activation of plant defence mechanisms (Hooker *et al.*, 1994). AM inoculated plant generated various compounds such as phytoalexins, enzymes of the phenylpropanoid pathway, chitinases, β -1,3-

glucanases, peroxidases, pathogenesis-related (PR) proteins, callose, hydroxyproline-rich glycoproteins (HRGP) and phenolics. AMF are found to be naturally associated with commercial plants and cereals (Sharma *et al.*, 2019). These compounds are related to plant's defence mechanisms. AMF initiated host defence responses which were subsequently suppressed disease infestations. A report showed that there was a minor change in the accumulation of defence related transcriptions in *Glomus intraradices* colonized roots as compared to un-inoculated controls (Gianinazzi and Pearson, 1992). However, peroxidase activity concerned with epidermal and hypodermal cells was enhanced in mycorrhizal roots, which could be a possible mechanism for higher resistance to root pathogens. The enhanced lignification of root endodermal cells by AMF colonization has been recorded (Houlton, *et al.*, 2018). Nevertheless, AMF symbiosis-specific genes control the expression of the genes concerned to plant defence during the establishment of AMF (Gianinazzi-Pearson *et al.*, 1996).

AMF are one of the most important components of many plant rhizosphere and play an important role in decreasing occurrence of plant disease (Akhtar and Siddiqui, 2008). The application of AMF as a biocontrol agent performed a vital role in plant resistance and display greater potential to protect bean plants against the infection with *F. solani* (Al-Askar and Rashad, 2010). The causal agent of bacterial wilt disease in tomato and many other vegetable crops is *Ralstonia solanaceum*. Tomato plants colonized by *Glomus mosseae* abridged infestation of *R. Solanaceum* (Vailleau, *et al.*, 2007). Control and management of *Heterodera avenae* on wheat using arbuscular mycorrhiza with oil cakes was done successfully (Sharma and Trivedi, 2014). Moreover, the positive results of *G. etnicatunium* and *G. margarita* spores were found against the *Verticillium* disease in brinjal (Lehmann and Rillig, 2015).

AMF are able to grow with *Trichoderma harzianum*, which is known as a bio-enhancer and have potential to suppress many diseases of oil palm. Results showed that, although, *T. harzianum* infused-compost (TC) gave highest yield, these mixed inoculum (TC and AMF) produced higher yield than AMF alone. Comparative efficacy of nematicides with AM fungi has also been tested against *Heterodera avenae* infecting wheat. Studies have been carried on concomitant effect of AM and neem products on nematode infected cereals like

wheat (Sharma, 2013; Pozo and Azcon-Aguilar, 2007).

Role of Am Fungi in Disease Resistance and Plant Defence

Mycorrhizal roots often exhibit very intense fungal colonization, both inter-cellularly and intra-cellularly, that can reach upto 90% of total root length. This observation has led Dangeard to coin the genus name *Rhizophagus* (greek word for "root eater"), based on the initial assumption that mycorrhizal roots were colonized by an aggressive pathogen (Rillig, *et al.*, 2018). We now know that most plants can potentially get prot from AM fungal colonization (depending on the right fungal partner and the environmental conditions), but it is still a mystery how plants can tolerate such high degrees of colonization without mounting a defence response, given that fungi in general (including AM fungi) contain and release many molecular signals (e.g., chitin oligomers) that can be recognized by plants, and that have shown to trigger defence responses in various plant species (Wan *et al.*, 2008; Boller and Felix, 2009).

It has therefore been proposed that AM involves the suppression of defence process. Indeed, plant mutant defective genes are required for symbiotic signalling and AM establishment often show characteristic defence responses upon infection by AM fungi (Kamel *et al.*, 2017). Indicating that these fungi have potent signalling molecules that trigger defence mechanism, and that these mechanisms are suppressed during normal AM development (Kloppholz *et al.*, 2011).

Although defence mechanisms in the host have to be attenuated to allow AM fungal infection and colonization of the roots, general defence also needs to remain active to cope with rhizospheric pathogens. In contrast, mycorrhizal plants often exhibit increased disease resistance (Sharma and Trivedi, 2001). Experiments with split root systems revealed that this effect is often systemic, i.e., the entire plant is protected against pathogens (Jung, *et al.*, 2012). This can involve generally improved plant health due to better nutrition, or a systemic induction of the defence status, known as systemic acquired resistance (SAR). Adding together, plants with AM fungi colonization are/ may be well geared up in advance to respond more rapidly and sturdier to pathogen attack, a phenomenon known as induced systemic resistance (ISR), or priming (Conrath, *et al.*, 2006). These protective effects of

AM are of great interest for sustainable strategies of plant protection (Cameron, *et al.*, 2013). Although priming is a systemic phenomenon, AM fungi are primarily employed to protect plants from soil-borne pathogens (Gao, *et al.*, 2020; Jung, *et al.*, 2012).

AM fungi and associated other microbes, can unswervingly impede with rhizospheric pest/pathogens either by straight competition for resources and space or by the secreting antimicrobial compounds that are toxic to them. Although the potential of AM fungi for plant protection is widely acknowledged, it should be noted that in certain cases, mycorrhizal crops have no benefits from AMF, or may even exhibit reduced growth and fitness (Jacott, *et al.*, 2017). It is tempting to speculate that this phenomenon may be related to breeding programs that targeted traits related to shoot architecture and yield, while root-related traits were ignored. While this does not necessarily prevent plants from becoming infected, it may have interfered with the regulatory mechanisms that ensure optimal metabolic coordination of both partners.

Mycorrhizal Effects on Soil Structure

Soil structure refers to soil particle aggregation as well as pore spaces. Maintenance of soil structure is of critical importance to the preservation of soil functions and fertility. Mycorrhizal fungi produce biological glue, glomalin which is a key player in soil aggregation via hyphal network production. AMF presence in the soil is indispensable to uphold substantial structural properties of soil. Thus, healthier soil structure results in: superior root development, added air permeability, elevated microbial activity and nutrient cycling, greater infiltration of water and water holding capacity and over all improved resistance to crusts, to compaction, to erosion.

An important service of AM fungi in natural as well as in agricultural contexts is the beneficial alteration of soil structure, reduction of soil erosion and nutrient leaching (Leifheit, *et al.*, 2014). The dense hyphal network of the highly ramified AM fungal mycelium creates a three-dimensional matrix that enmeshes and cross links soil particles without compacting the soil. A soil glycoprotein was identified as an additional important agent in the stabilization of soil aggregates (Vlèek and Pohanka, 2020; Singh, *et al.*, 2013). It is referred to as glomalin, because it is thought to be produced by AM fungi. Glomalin and glomalin-related soil proteins (GRSPs) are not a

dened gene product or chemically homogenous molecular species; rather they are a soil fraction that is dened by its extractability and immuno-reactive properties (Rillig, 2004). Glomalin is considered as resurgence in the review of literature; nonetheless, their source and purpose are not clear yet. Nevertheless, they represent an important determinant of soil quality and a very stable carbon sink with estimated half-life times in the range of several years up to decades (Rillig, *et al.*, 2001).

The collective effects of AM fungi on soil qualities also results in higher water retention capacity, which benets plant growth in addition to improved nutrient supply. The benets of AM fungi are particularly critical for plants in dry sandy soils in arid regions. These soils often show low fertility and are highly vulnerable to erosion by wind and rain. In such cases, plantings with mycorrhizal plants can be a sustainable way to counteract erosion and improve soil fertility (Gutjahr and Paszkowski, 2013).

The benecial effects of AM fungi against nutrient leaching operate at different levels. First, improved soil structure allows increased nutrient sequestration to the micro and macro-aggregates in mycorrhizal soil. Second, AM fungi take up nutrients from the soil solution, and thus nal mycorrhizal soils exhibit better retention capacity of the soil solution, thereby besetting at the same time the availability of nutrients and water to the plant. A detailed documentation of the benecial effect of AM fungi on plants under drought stress was reported for tomato. Reduced leaching from mycorrhizal soils has been documented in particular for P and N, but it conceivably also involves other mineral nutrients (Cavagnaro, *et al.*, 2015). Taken together, AM fungi integrate the nutrient uxes in the soil by generating closed nutrient cycles, thereby promoting long-term soil fertility (Cameron, *et al.*, 2013).

Use of Am Fungi in Renaturation, Aforestation, and Landscaping

Renaturation and aforestation are measures to stabilize degraded and eroding land surfaces. In particular in arid and semi arid regions, young trees are extremely vulnerable to abiotic stresses (drought, heat, nutrient starvation), in particular at early stages until they have established a deep root system that allows them to access ground water reserves. This critical phase can be overcome with mycorrhizal inoculation of the trees before planting. For example, the Moroccan argan tree, the fruits of

which are used to prepare the precious argan oil (El Abbassi, *et al.*, 2014). are endangered in their original areas of distribution due to overuse, despite of their protection as UNESCO biological reservation. Argan reforestation requires that young plantlets raised in nurseries are planted out, and that they quickly adapt to the dry climate of the native range of these trees. Mycorrhizal inoculation majorly improves growth, health and development of young argan trees, thus increasing their vigour and survival chances after planting.

A similar case is represented by the use of a mixture of indigenous AM fungi for the inoculation of young Cypress trees. In this study, only AM fungi isolated from the natural site of *C. atlantica* were used, thereby increasing the chances to employ fungi that are well adapted to drought and to *C. atlantica*, and thus avoiding introducing new AM fungal species with unpredictable effects on the local environment. AM inoculation not only increased plant growth, but also increased survival of the trees in the dry native conditions. This latter point is perhaps even more important than the growth promotion, because it renders reforestation efforts more sustainable (Lahcen *et al.*, 2007) another interesting example is stabilizing sand dunes by planting of the drought-tolerant mesquite tree (*Prosopis juliflora*), which increases mycorrhizal communities in sand dunes. On the other hand, the mesquite trees get benefit from AM colonization. Hence, AM symbiosis can be a critical component in framing strategies to protect vulnerable sandy soils against erosion, and to improve their fertility as well.

Use of AMF for Phytoremediation of Metal Polluted Soils

Soil microorganisms are known to play a key role in the mobilization and immobilization of metal cations, thereby changing their availability to plants. Along with other validated microbes, AMF are among the most common soil microorganisms helping in remediation/degradation and constitutes an important functional component of the soil-plant system occurring in almost all habitats and climates, including disturbed soils (Rivero *et al.*, 2018; Kedia and Sharma, 2015). Degraded soils do, however, suffer from changes in diversity and abundance of AM fungal populations. More explicitly, it was observed that AMF cocurrence can be influenced by heavy metal toxicity, but in numerous cases mycotrophic plants have shown good AMF colonization in heavy metals contaminated soils.

Many reports concerning this have quantified spores and estimated root colonization *in situ*. Occurrence of metal tolerant AMF in heavy metal polluted soils has been reported by others. In last decade, research interest have been developed trying to understand the foundation underlying AMF tolerance and adaptation to heavy metals in contaminated soils, in view of the fact that for aforestation/restoration/bioremediation programs this could aid the administration of these soil microorganisms.

Role of AMF in PAH Polluted Environments

Polycyclic Aromatic Hydrocarbons (PAH) are hydrophobic organic molecules which consists of two or more fused benzene rings. A selection of 16 PAH is commonly quantified for characterization and monitoring of these pollutants and around 200-300 PAH compounds along with their derivatives are usually extracted and could be identified in contaminated soil test sample. The concern for PAH pollution is derived from their ubiquitous distribution, their recalcitrance towards degradation and their proven or suspected mutagenic properties (Sharma and Pareek, 2014).

The origin of PAH may partially be natural (organic residues after tire), or anthropogenic (mainly processing and incomplete combustion of fossil fuels). Thus, oil spills and industrial sites. e.g., for coke distillation etc. commonly give rise to extreme pollution event; for which physical, chemical and biological remediation strategies are employed for cleanup. These include, among others, bio-venting, land farming, bio-augmentation and phytoremediation. This is only possible and applicable when physical attributes and pollution levels in soil may allow the plants to grow. This is a cost-effective, eco-friendly and efficient management that concurrently restores an ecosystem, limits soil erosion and improves over all esthetical impression of a polluted site (Wilson, *et al.*, 2016).

AMF may be directly or indirectly effects PAH degradation in the rhizosphere. As PAH are not absorbed by plants and are metabolized intracellularly, all degrading activity would take place in soil or inside soil organisms other than AM. Moreover, the only likely straight effect of AMF on degradation of PAH would be via enhanced production of extracellular peroxidises, as AMF have poor saprophytic ability. Tortuous effects would be owed to changes in the microbial community e.g. due to stronger competition for

nutrients, minerals, straight synergistic or antagonistic belongings of AMF or pattern changes in root exudates.

Mycorrhizae-assisted Remediation (MAR)

Mycorrhizae-assisted remediation is a facet of bioremediation that employs use of mycorrhizae for the treatment/remediation of polluted soils. Mycorrhizal fungi are also able to detoxify toxic substances; hence they have been used for the remediation of both organic and inorganic pollutants in soils. Remediation of polluted soils can be done by the two common types of mycorrhizae – ectomycorrhizae (ECM) and arbuscular mycorrhizae (AM). However, AM is used in most remediation exercises because it colonizes almost all types of plants unlike ECM that colonizes mostly woody species.

Mycorrhizae cannot exist without a plant; therefore MAR can be described as a modified form of phytoremediation that exploits the benefits derived from mycorrhizal fungi. Phytoremediation like as phytoextraction and phytostabilization are the principal techniques it uses. Nonetheless, it is quite dissimilar from phytoremediation for the reason that remediation can be attained at a faster pace because the area covered by plant roots, through the fungi hyphae, in MAR is greater than the area covered in phytoremediation. The advantages of MAR clean-up includes; it is a natural process carried out *in situ* thus eliminate transporting polluted soils, it is environmentally friendly; remediation of a wide range of organic and inorganic pollutants; it enhances the re-vegetation of a soil later; increased nutrient and water uptake, disease resistance and soil stabilization (Chibuike, 2013).

Mass Production of AM Fungi

The AM fungi being not host specific can infect and colonize any plant species but the level of AM colonization and its effect on plant growth and vigour can vary with different host-endophyte arrangement. Cultures of AM fungi on plants growing in disinfected soil have been frequently used technique to increase propagule numbers/inoculum. A highly susceptible host plant should be used for its mass production. Host plant should produce plentiful roots rapidly and bear the high-light environment setting that is required for the AMF to multiply quickly. Trap plants should be screened to ensure that maximum levels of

inoculum were achieved, later (Machado *et al.*, 2017). Pot culture technique could also be employed to produce large quantities of the inoculum. Plants with mycorrhizal associations predominate in most natural eco systems, so as such inoculum of mycorrhizal fungi is present in most soils. The inoculum quantity of AM fungi whether compatible with a host plant in soils can be measured by bioassay experiments. In these experiments, seedlings are grown in intact soil cores or mixed soil samples for sufficient time to allow mycorrhizae to form, and then roots are sampled, processed and assessed to measure mycorrhizae formation.

Numerous host plants like Cenchrus grass (*Cenchrus ciliaris*), Sudan Grass (*Sorghum bicolor var. sudanense*), bahia grass (*Paspalum notatum*), Clover (*Trifolium subterraneum*), Strawberry (*Fragaria sp.*), Sorghum (*Sorghum vulgare*), Maize (*Zea mays*), Onion (*Allium cepa*) and Coleus (*Coleus sp.*) have been used for their suitability to multiply AM fungal inoculum. Various solid growth medium such as soil, clay, peat, vermiculite, sand, perlite or composted barks in combination or individually, are widely used to grow the fungus with the host plant. However, there are some reports in which AM colonization has been detected in Chenopods in the field and pot culture.

Mercantile Use of AM Fungi

The multiple benefits of AM have raised opportunities for their commercial application. Consequently, the AM-related markets grew considerably during the past few decades, with increasing numbers of products and market volume (Vosatka, *et al.*, 2008). However, due to the fact that most of the AM-related industry consists of privately owned relatively small firms, public information about the dynamics of market shares is quite scarce.

Since the 1990s, the number of companies selling mycorrhizal products has increased considerably. On a global scale, the main players are located in North America, Europe, Asia, and Latin America. In the domain of the Americas, the main markets include United States, Canada, Mexico, Brazil, Argentina, Colombia, and Chile. The Asia region is mainly dominated by India, followed by China. The Indian market itself has seen an outstanding growth rate during the last decade. One of the reasons is the promotion of mycorrhizae based biostimulants by the Indian government and the actions from many private organizations. The European market

represents one of the leading markets for mycorrhizal bio-stimulants. In Europe itself, the number of firms producing and selling AMF-products has increased from less than 10 firms in the late 1990s, to more than 75 firms in 2017. Most of the European companies are found in Germany, Italy, Spain, the United Kingdom, France, Netherlands, Czech Republic, Austria, Belgium, Estonia, and Switzerland.

CONCLUSION

Arbuscular mycorrhizal fungi promote many aspects of plant life, in particular improved nutrition, better growth vigour, stress tolerance, and disease resistance. In addition, the hyphal networks of AM fungi improve soil characters such as soil particle aggregation thereby improving the resistance of soil toward erosion by wind and water. These multiple benefits of AM fungi translate into significant ecological services in natural contexts. AMF colonized with several cereal, vegetables, fruits and industrial crops. However, mycorrhizae exhibited better performance in low P added soil. The benefits derived from mycorrhizal fungi make MAR a suitable method for the clean-up of soils whose intended use is enhanced crop production, food security and sustainable agriculture. MAR effectively detoxifies both organic and inorganic pollutants. However, more research is needed in order to harness the benefits of these advantageous inoculants on crops which will help to raise farmers' economic status worldwide.

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