

ARBUSCULAR MYCORRHIZAE: A DIVERSE PERSONALITY

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Manuscript received: December 30, 2005; Reviewed: June 7, 2006; Accepted for publication: August 15, 2006

ABSTRACT

Arbuscular mycorrhizae (AM) are beneficial symbionts for plant growth. They are associated with higher plants by a symbiotic association, and benefit plants in uptake of phosphorus nutrients, production of growth hormones, increase of proteins, lipids and sugars levels, helps in heavy metal binding, salinity tolerance, disease resistance, and even in the uptake of radionuclides. Mycorrhizal genes also applicable in improvement of crop plants, due to their delivery in to plants, by a process called, particle bombardment. The combined association of mycorrhizal fungi and Rhizobium, with legume plants, as a symbiotic association, increased the beneficial aspects comparatively more than their single associations with the host plants. This review focuses on all beneficial aspects of AM fungi, regarding plant growth.

KEY WORDS: mycorrhizae, association, inoculum, Glomus, uptake.

Introduction

Perhaps the most widespread and certainly significant mutualism between plants and fungi is the root symbiosis, termed arbuscular mycorrhiza (AM). These fungal endosymbionts, having nearly universal in their association with flowering plants, including agriculturally important crop species [27]. The fungi enter cortex of roots to obtain carbon from their host plants, while assisting the plants with the uptake of phosphorus and the other mineral nutrients from soil [4]. This association is beneficial to plants because, phosphorus is a major essential element for growth and development. The other functions attributed to AM fungi include production of plant growth hormones, protection of host roots from pathogens, uptake of heavy metals, salinity tolerance, uptake of radionuclides, and protect plants from radio activity [56, 57]. In this review, we focus on the association of arbuscular mycorrhizal fungi with some host plants, and its benefit to the hosts in various aspects.

AM Fungi - a History of Early Origin

A discovery of arbuscules in *Aglaophyton major*, an early devonian plant provides unequivocal evidence that mycorrhizae were established more than 400 million years ago [52]. Mycorrhiza is the mutualistic symbiosis (non-pathogenic association) between soil borne-fungi with the roots of higher plants [50], revealed that they are found in a wide range of habitats usually in the roots of angiosperms, gymnosperms and pteridophytes. They also occur in the gametophytes of some mosses, lycopods and psilotales, which are rootless [42]. Recently they were also reported in aquatic plants, by Quilambo [50]. During symbiotic interactions, the AM fungi support to its host plants by many beneficial aspects, including phosphorus uptake and helps for plant growth.

During symbiotic interactions, AM fungi germinate and develop a presymbiotic mycelium with limited growth before coming into contact with the host root to form appressoria [22]. After appressorium formation, roots can be colonized into two different ways [62]. The arum-type of colonization, which is the most studied form, is characterised by intercellular spread of the hyphae until they reach the inner cortex, where the plant cell wall is penetrated and the fungus extensively ramifies to form an arbuscule. In paris-type of colonization, fungal development is only intracellular, and arbuscules are formed from hyphal coils. Numerous modifications have been observed in host cells during the development of arum-type arbuscules [67]. Cytoskeleton elements are rearranged, the nucleus increases in size, and plastids

are reorganised. H_2O_2 is accumulating and changes occur in the membrane system of arbuscule-containing cells. The golgi apparatus is highly active and the plant plasma membrane extends to form a novel periarbuscular membrane (PAM) which closely surrounds the fungal hyphae. Increased ATPase activity can be detected at the PAM, which is probably the prerequisite for the uptake of nutrients from the matrix between the fungus and the plant [63].

Preparation of AM Inoculum

Various techniques are available for the production of AM inoculum in an almost sterile environment, through nutrient film techniques, aeroponic culture systems, and root organ cultures [51]. However, for large-scale production of inoculum, the available and widely practised method is the traditional pot-culture techniques employing trap plants [9]. Potty [49] reported that AM fungi *Glomus mosseae*, multiplied in cassava (*Manihot esculenta*) tuber peel and the number of spores ranged from 3 to 4/cm², suggested that the peel could be used for mass multiplication of AM fungi. Ganesan and Mahadevan [20] claimed that hyphae, arbuscules and vesicles of *G. aggregatum* developed on the surface of cassava tuber and that could be used as inoculum. Recently, Selvaraj and Kim [58] introduced sucrose-agar globule with root-exudates (SAGE), as a source of inoculum to increase the production of AM fungal spores. It was observed that the SAGE showed a higher percentage of root colonization (about 10% more), and increases in the number of spores (about 26%) and dry matter content (more than 13%) for the AM fungal spores than their soil inoculum.

Even though many kinds of techniques available for production of inoculum of AM fungi, the method followed most common in practise is the traditional pot-culture method, using a host plant. In all the methods, only a few spores (5 to 10 spores) are used for the production of AM fungal inoculum. The process of inoculation is done in host plants, through their root region. After inoculation, the AM fungi develop its association with the root cells and then do its further beneficial activities with the host.

Uptake of Phosphorus Nutrients

Phosphorus is one of the key macronutrient required for plant growth and metabolism. It plays an important role in energy transfer through the formation of energy-rich phosphate esters and is also an essential component of macromolecules such as nucleotides, phospholipids and sugar phosphates [40]. Much of the inorganic phosphate applied to soil as a fertilizer is rapidly converted to unavailable forms with low solubility. Soluble P is released

from insoluble phosphates by a variety of solubilization reactions involving rhizosphere microorganisms [31]. Mycorrhizal plants can take up more phosphorus than non-mycorrhizal plants, mainly from the same soluble phosphate pool. Inoculation with phosphate solubilizing microorganisms (PSM) may help to solubilize native soil phosphate, as well as phosphorus from rock phosphate. Soluble phosphate released by the activity of phosphate-solubilizing microorganisms (PSM), can actively taken up by mycorrhizal roots [31].

The rate of absorption of phosphate by growing roots is much higher than the rate of soil phosphate diffusion, which results in the formation of a phosphate depletion zone at the root system level and consequently limits the supply of phosphorus to the plant. The growing plant root creates a phosphate depletion zone caused by contrastingly high plant phosphate uptake and low soil-based phosphate diffusion rates. The extra-radical mycelium of arbuscular mycorrhizal fungus grows far beyond the depletion zone, reaching a new pool of soluble phosphate [61].

The major function of AM fungi is phosphate uptake, because it encodes a phosphate transporter gene. Harrison and Van Buuren [24] investigated a process for phosphate transport by identifying a complementary DNA (cDNA) that encodes a transmembrane phosphate transporter termed GvPT from *G. versiforme*. The function of GvPT protein was confirmed by complementation of a yeast phosphate transport mutant. Expression of GvPT was localized to the external hyphae of *G. versiforme* during mycorrhizal association, this being the initial site of phosphate uptake from the soil. Chellappan [10] isolated a phosphate transporter gene from *G. deserticola*, also showed the role of AM fungi, in the uptake of phosphate.

Production of Plant Growth Hormones

Roots colonized by AM fungi are often thicker and carry fewer root hairs. Such changes in morphology are expected to be under phytohormonal control [60]. Abscisic acid (ABA) was found to be considerably enhanced in both roots and shoots of AM plants, as compared to non-mycorrhizal control [14]. Indirect ELISA tests with polyclonal antibodies against ABA showed that the level of this phytohormone is at least twenty times higher in spores and hyphae than in roots of maize at all stages of plant development [14].

It is unlikely that ABA synthesized originally is by the maize cells, since AM fungi would then have to enrich this phytohormone from the apoplasm, by active transport. It is more probable that the fungus exerts control over the morphology of the roots and ABA plays an important

role in this. ABA is involved in the regulation of soluble fluxes with in plants, which could also happen in AM fungal symbiosis [17]. Also, an increase of IAA (Indole acetic acid), gibberellin and cytokinin level was observed in *G. fasciculatum* inoculated *Prosopis juliflora*, recorded by Selvaraj [60], showed the influence of AM fungi, *G. fasciculatum*, on increased level of growth hormones. Barea and Azcon-Aguilar [6] also noted that in axenic experiments, mycorrhizal fungi produced auxin, gibberellin and cytokinin-like substances and stimulated plant growth.

Enzyme Activities

Increased peroxidase is one of the most widespread biochemical activities in diseased and injured plant tissues. Peroxidases has an important function in secondary cell wall biosynthesis by polymerizing hydroxy and methoxy cinnamic alcohols into lignin [23] and is also associated with deposition of suberin in plant cell walls around the lesions [71]. Spanu and Bonfante-Fasolo [65] measured the cell wall bound peroxidase in *Allium porrum* during root growth and development of *G. versiforme*. An initial stage of fungal infection, the enzyme activity was maximum and decreased in highly colonized roots. Pacovsky et al. [46] studied peroxidase activity in *Phaseolus vulgaris* infected by *G. etunicatum*, and found that peroxidase activity increased in the mycorrhizal plants.

Phosphatases of the mycorrhizae are both specifically induced in the presence of *Glomus* spores and are sensitive to the level of phosphate in the environment [45]. Under phosphorus starvation plant cells produce a major acid phosphatase with a pronounced preference for phosphoenol pyruvate [18]. Mac Donald and Lewis [38] cytochemically demonstrate the presence of acid phosphatase in *G. mosseae*. Selvaraj [60] found that due to inoculation of AM fungi, *G. fasciculatum*, acid phosphatase activity was increased in leaves and roots of *P. juliflora*.

Pacovsky et al. [45] revealed that the increase in polyphosphate hydrolase in AM fungi associated root is interesting since it raises the possibility that the fungal endophyte may enhance phosphate availability in the rhizosphere. Typically, plants do not contain their own polyphosphate hydrolase; rather, they rely on the activity of soil microorganisms to release free phosphate from mineral or organic P resources. Tisserant et al. [68] observed on histochemical tests revealed the presence of alkaline phosphatase in *Glomus* infected roots of *A. porrum* and *Platanus acerifolia*. The presence of AM specific alkaline phosphatase activity in *A. cepa* and

P. occidentalis plants inoculated with *G. mosseae* has been reported [21]. Selvaraj [60] reported an increased level of alkaline phosphatase activity in *G. fasciculatum* inoculated roots of *P. juliflora*.

A DNA-hybridization experiment with the digoxigenine labelled PCR-segment and DNA isolated from about 0.5 million *Glomus* spores confirmed that mycorrhizal fungus possesses a nitrate reductase gene [30]. Selvaraj [60] also observed an increase of nitrate reductase activity in *G. fasciculatum* inoculated *P. juliflora*, treated with tannery effluents.

Rate of Photosynthesis

Reports from Selvaraj [60] showed an increase of photosynthetic activity in leaves of *P. juliflora*, inoculated with *G. fasciculatum*. There was an improvement in chlorophyll contents (chlorophyll a, b and total chlorophyll) also noticed in the leaves of *P. juliflora*, inoculated with *G. fasciculatum* [60].

AM fungi have been shown to increase stomatal conductance and photosynthesis after water stress of rough lemon [37] and to increase both transpirational and photosynthetic rates as well as chlorophyll concentrations in the grass *Bouteloua gracilis* [2]. It was observed that bundle sheath chloroplasts were more numerous and that the veins and mesophyll cells of mycorrhizal finger millet were larger than those of non-mycorrhizal plants [35].

Levels of Sugars, Lipid, Amino acids and Protein Content

Mycorrhizal plants showed an increased level of soluble sugars. Selvaraj [60] reported soluble sugars in both leaves and roots were increased in *G. fasciculatum* inoculated *P. juliflora*. Same et al. [53] investigated that the percentage of root infection by *G. fasciculatum* was closely correlated with concentrations of soluble carbohydrates in the inoculated roots. Mycorrhizal inoculation involves in the increase of reducing sugars in the inoculated plants. Increased levels of reducing sugar [64] in *Allium porum*, *Citrus aurantium*, *Sorghum bicolor* var. *sudanensis* and *Lycopersicon esculentum* [66] were found on *G. fasciculatum* inoculation.

Mycorrhizal inoculation increased lipid level in the inoculated plants. Cooper and Losel [13] showed that roots associated with *G. mosseae* contained significantly more lipid than the non-inoculated roots. The mycorrhizal roots of *A. cepa*, *Trifolium repens* and *Lolium perenne* contained large amount of total lipid than non-mycorrhizal roots. Pacovsky and Fuller [48] demonstrated that four soybean cultivars associated with *G. fasciculatum* had higher level of fatty acids in both leaves and roots than non-mycorrhizal plants. Report from Selvaraj [60] also

revealed a higher level of lipid content in AM fungi *G. fasciculatum* inoculated plants of *P. juliflora*.

Mycorrhizal inoculation increased the level of total amino acids in the inoculated plants. Nemeč and Meredith [43] found that *G. etunicatum* inoculated *Citrus limon* leaves had higher total amino acids than control. Selvaraj [60] also reported increased level of total amino acids in *P. juliflora*, inoculated with *G. fasciculatum*. Protein content also increased in arbuscular mycorrhizae inoculated plants. It was revealed that *G. fasciculatum* inoculated, tannery effluent treated *P. juliflora* showed an increase of protein content in both leaves and roots than the control; where as plants treated with tannery effluent alone showed least protein content due to the absence of mycorrhizal influence [60]. Higher protein content in mycorrhizal roots than in non-mycorrhizal root extract was also observed by Arines et al. [3] in red clover.

From the observation of Selvaraj [60], the protein profiles of leaves of *P. juliflora*, inoculated with *G. fasciculatum* showed much of difference between other treatments. In plants treated with tannery effluent alone, many of the protein bands were not found. In *G. fasciculatum* inoculated effluent treatments some bands were missing, when compared with the *G. fasciculatum* inoculation, without effluent treatment. It was observed that the polypeptides with 16, 17, 18, 22 and 30 kDa were found only in VAM roots and were considered to be VA mycorrhizins [3, 47]. In the observation of Selvaraj [60], presence of the polypeptide, 30 kDa in AM + Effluent treatment and 22 kDa in AM + *Rhizobium* + Effluent treatment, confirmed the observations of earlier reports of Arines et al. [3] and Pacovsky [47].

Protection of Host Roots from Pathogens

Increased accumulation of phenols in roots caused resistance to pathogen in AM fungi inoculated plants. It was reported that an increase in phenols of roots of *Arachis hypogea* colonized by *G. fasciculatum* [34]. Codignola et al. [12] found that *G. versiforme* inoculated *A. porrum* showed a high level of phenols. The increased level of orthodihydric phenol was also correlated with resistance to pathogen. Benhamou et al. [7] suggested that the deposited phenols may act as a barrier to pathogen. Mahadevan [39] reported that the contents of total phenol and orthodihydric phenol would be increased in both roots and leaves of the mycorrhizal plants. The increase in total phenols in AM inoculated plants could be attributed to triggering of pathways of aromatic biosynthesis.

Uptake of Heavy Metals

Among soil microorganisms, mycorrhizal fungi are the

only ones providing a direct link between soil and roots, and can therefore be of great importance in heavy metal availability and toxicity to plants [36]. The toxicity of metals in soil depends upon their bioavailability, defined as their ability to be transferred from a soil compartment to a living organism [29].

High metal concentrations in soil are toxic to bacteria and fungi. In mine spoils heavily polluted with metals (up to 8.3% Zn and 863 $\mu\text{g g}^{-1}$ Cd). Diaz and Honrubia [16] used *Medicago sativa* to show that mine spoils and waste containing Zn and Pb (upto 236 and 456 mg Kg⁻¹ DTPA extractable Zn and Pb, respectively) had a mycorrhizal infection potential, although the number of AM fungal spore was lower than adjacent soil, not altered by mining activity. Turnau et al. [69] revealed that in *Oxalis acetosella* plants colonizing acidic forest soils with low pH, treated with Cd, Zn and Pb containing industrial dusts showed even higher mycorrhizal colonization than in non-treated soils. The fact that mycorrhizal colonization occurred in these observations suggests metal tolerance of AM fungi.

Since AM fungi cannot be cultivated without a host plant, it is more difficult to demonstrate the intrinsic metal uptake by their hyphae. Using culture systems which separate extraradical hyphae from roots, it has been shown that extraradical hyphae can accumulate and translocate ⁶⁵Zn, to a degree that may differ between species [8]. Adding ¹⁰⁹Cd to a hyphal compartment, Joner and Leyval [28] showed that extraradical hyphae may transport Cd from soil to roots. Mycorrhizal plants are of great interest since mycorrhizae can bind metals and limit their translocation to shoots [56]. Mycorrhizal fungi appear to partially protect plants against the toxicity of heavy metals. On the other hand, the host plant may give the fungus a selective survival advantage at a contaminated site. This mutual benefit would make mycorrhizal association superior to the application of single organisms.

Relatively large amounts of extraradical hyphae can be produced and separated from the roots, which should allow study of metal absorption and uptake by AM hyphae, transfer to the root and translocation to the shoot. Haselwandter [25] proposed that a metal-resistant plant breed should still be susceptible to mycorrhizal symbiosis and when colonized by a metal-resistant mycorrhizal fungus would be of great value for the rehabilitation of metal contaminated soils. Selvaraj [60] observed that due to the inoculation of *G. fasciculatum*, in tannery effluent treated *P. juliflora* the uptake of heavy metals such as Cd, Cr, Zn were highly restricted from the soil to the aerial parts, because of binding of those heavy metals in extraradical hyphae of *G. fasciculatum*, thus caused survival of the experimental host, *P. juliflora*

in the polluted soil.

Salinity Tolerance

In saline and sodic soils, drainage is poor and salt accumulates on the surface of soil, thus adversely affecting plant growth. Application of AM fungi is the simple way to improve survival of the vegetations, because these organisms are important components to long-term health and stability of maritime sand dunes [33]. AM fungi in dune systems contribute to binding of sand grains into large aggregates and to improving soil structure, factors that can influence plant succession [59]. In observation on *P. juliflora*, grown on saline soil inoculated with different AM fungi, as field inoculation, it was found that AM fungi, *G. macrocarpum* as the most efficient saline tolerant AM fungi, with maximum percentage of AM association in root and more beneficial effect on plant growth. This observation showed that inspite of saline toxicity, AM fungi are efficient in tolerating the saline-stressed ecosystem [59]. In addition, woody legumes, *P. juliflora* in particular, are recognized as useful for revegetation of water-deficient, low-nutrient environments, due to their ability to form higher percentage of symbiotic association with *Rhizobium* bacteria and AM fungi, which improves nutrient acquisition and helps plants to become established and cope with stress situation. Moreover, woody legumes generally exhibit a considerable degree of dependence on mycorrhizae to thrive under stressed situations [59].

Uptake of Radionuclides

In an atomic power station, products of nuclear fission reactions such as cesium, ¹³⁷Cs and strontium, ⁹⁰Sr are regularly released in to the environment as a result of weapons testing, nuclear power production and nuclear fuel reprocessing [26]. Plants absorb these ¹³⁷Cs and ⁹⁰Sr less efficiently than their nutrient analogues potassium and calcium, respectively [72]. It was explained by White and Bradley [70] that K⁺, the analogue of ¹³⁷Cs, moves into root by a symplastic pathway, and Ca²⁺, the analogue of ⁹⁰Sr, moves into root by an apoplastic pathway. As explained, both ¹³⁷Cs and ⁹⁰Sr are taken up from soil solution by plant as K⁺ and Ca²⁺, as they are similar to those cations in chemical properties [70].

Since AM fungi is beneficial for uptake of nutrients, and also for plants to survive, even from a disturbed soil after radionuclide deposition [1], a few study sites were selected in the constructed power house area of Kudankulam and it was carried out to understand the occurrence of AM association, and its influence on survival of vegetations in three disturbed sites of Kudankulam [57]. The two experimental plants *Phyllanthus niruri* and *Eclipta*

alba, inoculated with selected AM spores revealed that *G. fasciculatum* as the most efficient AM fungi with maximum AM association in root, and was more beneficial for plant growth. It was noticed that in spite of growth disturbance, AM fungi inoculated plants were efficient in tolerating the endangered ecosystem. Similar results had been reported earlier [41]. The two experimental host plants, *Phyllanthus niruri* and *Eclipta alba* showed more uptake of K^+ and Ca^{2+} in the root of AM inoculated plants, revealing the influence of AM fungi for the plants to take up more nutrients like potassium and calcium from the soil area. Even the mechanisms by which ^{137}Cs and ^{90}Sr are taken up by plant roots are not completely understood, previous studies [70, 72] revealed that Cs^+ is absorbed as K^+ by the K^+ uptake system of the root.

This evidence showed that K^+ uptake system strongly suppresses Cs^+ uptake, and K^+ is efficiently transported by an isolated high affinity K^+ uptake transporter of root cells of plants. Since ^{137}Cs and ^{90}Sr behave like potassium and calcium, respectively, the soil characters showed its activity to hold the radionuclides, and prevent radioactivity. Chances are greater for AM fungi to absorb and translocate K^+ of the radionuclide cesium and Ca^{2+} of strontium, like usual potassium and calcium absorption and translocation [1, 57]. Thus, through the help of AM fungi and the soil's nature to hold the radionuclide to prevent the expression of radioactivity, chances are greater for the vegetations to survive in the disturbed ecosystem in a better way. There is a very strong circumstantial evidence therefore that AM fungi would enhance uptake and recycling of radionuclides, particularly ^{137}Cs and ^{90}Sr [57].

Application of Mycorrhizal Genes

Phosphate is one of the important minerals that play a vital role in human and animal health. Plants in general have the ability to uptake mineral nutrients only in certain forms. Therefore, it is smart to have the transporter genes in plants for efficient nutrient transport. Phosphate-transporter gene has been identified in *G. versiforme* [24].

Several techniques have been developed for gene transfer to microorganisms, plants and animal cells. Some systems utilize biological vectors such as retroviruses or *Agrobacterium* to accomplish gene transfer [32], others rely on chemical (calcium phosphate precipitation, polyethylene glycol or liposomes) or physical means (microinjection, electroporation or particle bombardment) to facilitate DNA transfer across cell membrane. Among the techniques, *Agrobacterium*-mediated transformation technology is inexpensive. But, certain crop plants are

recalcitrant. Those cases are not fit for *Agrobacterium*-mediated transformation. So, as an alternate technology, particle bombardment was developed primarily to overcome barriers in genetic transformation of plant species [54]. According to Christou [11], the tissues dispersed easily and proliferated rapidly in liquid culture to produce high-quality embryogenic suspension cultures in which majority of the cells are totipotent. These had been identified as ideal target tissues for use with direct gene transfer systems, because, they maximize the probability of the insertion and integration of the genetic material into large number of morphogeneical competent cells.

A protocol was established for the introduction of DNA into embryogenic suspension-derived tissues of cassava (*M. esculenta*, grown as an important calorie food source in tropical and subtropical areas), via microparticle bombardment, for selection of genetically transformed cells, and regeneration of fully transgenic plants, from the genetically transformed cells [55]. Chellappan [10] cloned phosphate transporter gene from *G. deserticola* has been successfully integrated into cassava plants by particle delivery system. The benefit get from this transgenic plants obtained from particle delivery system might be expected to uptake more phosphorus, and other mineral nutrition transport, induce the production of more growth hormones, more influence on salinity tolerance, heavy metal tolerance, disease resistance, and also uptake of radionuclides, than the non-mycorrhizal plants, and if so, it can be simply called as, transgenic mycorrhizal plants [10]. So, simply, mycorrhizal genes are highly applicable in improvement of crop plants.

Symbiotic Association with Rhizobium

Woody legumes are useful for revegetation of water-deficient ecosystems that have low availability of N, P and the other nutrients [15]. The scarcity of available phosphorus and the imbalance of trace elements in desertified ecosystems actually limit legume establishment and nitrogen fixation. But, when associated with mycorrhizae, it was found to increase the establishment of legume [5]. Moreover, woody legumes exhibit a considerable degree of dependence on mycorrhizae to thrive in stressed situations [44]. Arbuscular mycorrhizae are by far the most widespread in nature and the most common natural association makers with the nodulated nitrogen fixing legumes [5].

After forming symbiotic association with legume roots, AM fungi develops an extraradical mycelium that links the roots and the soil environment and helps the plants to use more efficiently soil nutrients, particularly those that

diffuse slowly towards the root surface, such as phosphate and trace elements [5]. In addition, the symbiosis enhances the ability of plant to become established and cope with stress situations (nutrient deficiency, drought, trace element imbalance, soil disturbance), which are typical in desertified situations [5].

Ferrera-Cerrato and Villerias [19] reported that nitrogen content increased higher in plants inoculated with *Glomus* species than in those inoculated only with *Rhizobium*. The largest nitrogen content corresponded to the treatment with both *Glomus* and *Rhizobium*. Selvaraj [60] reported that in *G. fasciculatum* and *Rhizobium* inoculated *P. juliflora*, treated with tannery effluent showed a higher dry weight, increased rate of photosynthesis, higher protein content, increased sugars, lipid and amino acid levels, increased activity of enzymes catalase, peroxidase, phosphatases, nitrate reductase, increased level of growth hormones IAA, gibberellin and cytokinins, higher N, P, K and Ca, Fe, Co, Mo levels in root and shoot, than their single inoculations. Also, the translocation of heavy metals such as Cd, Cr and Zn are highly restricted in the extraradical hyphae of AM fungi, in dual inoculated treatments, when compared with the single inoculations. Thus, the combined symbiotic association of AM fungi with *Rhizobium* benefits more to the plants, when compare with their single symbiotic association [60].

Conclusions

Arbuscular mycorrhizal (AM) fungi benefit its host plants in the following aspects. Plants receive supports from AM fungi, with the help of its symbiotic association, in the aspect of uptake of phosphorus, and other nutrients, enhancement of growth hormones, increase of protein content, increase of lipid, sugars, amino acid levels, increase of tolerance to heavy metals, increase of salinity tolerance, and resistance to root-borne pathogens. Also, AM fungi can helps in uptake of radionuclides in a disturbed ecosystem with radioactive elements. The delivery of mycorrhizal genes into plants by particle bombardment, and the plant may act as a transgenic mycorrhizal plant. The benefit of combined association of AM fungi and *Rhizobium* is comparatively more to the host plants, than their single associations with the host plants, especially in legume member hosts.

Acknowledgment

Financial support from Department of Environment, Government of India, to carry out work from the Centre for Advanced Studies in Botany, University of Madras, Chennai, India, is gratefully acknowledged.

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