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RESEARCH ARTICLE

SYNERGISTIC INTERACTIONS OF AM FUNGI WITH ESSENTIAL GROUPS OF MICROBES IN PLANT GROWTH PROMOTION

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ABSTRACT

The exploitation of AM fungi is highly significant to massive crop improvement programmes. It is important to withstand drought in the nursery and in the field. Improved growth of many crops, resistance of plants to drought stress and root pathogens due to application of AM fungi was emphasized by many workers. Considerable research efforts have been made to demonstrate that Mycorrhizal and N_2 fixing symbionts benefit the plant growth and these two symbionts can be used in agriculture to improve nursery quality and subsequent growth in the field. Rhizosphere microorganisms influence many chemical reactions by way of their metabolites and AM fungi play a crucial role in affiliating both microbial and plant functions as mediators of exchange between them. Because of the added advantage by the interaction between AM and different groups of microbes, Mycorrhizal technology has assumed greater relevance in crop production. The practical application can lead to potential increase in survival and growth rates of crops. This paper traces synergistic interactions of AM with other microbes that promote plant growth.

INTRODUCTION

Many microorganisms form the symbioses with plants that range from parasitic to mutualistic. Among this the most widespread mutualistic symbiosis is the arbuscular mycorrhizal association. Arbuscular mycorrhizal (AM) symbiosis occurs between the fungi of the Glomeromycota (Schubler *et al.,* 2001) and the majority of the terrestrial plants. The phycobiont correspond to 80% of plant species and this association involves an intimate relationship between plant roots and fungal hyphae with neighboring soil microbes. This mutualism is manifested in bidirectional nutrient exchange: the fungus is nourished by plant photosynthates and plant mineral nutrition particularly phosphate is enhanced by the fungus (Smith and Read, 1997). AM fungi are obligate biotrophs, depending on living root tissue for carbohydrate supply to complete their asexual life cycle. AM fungi receive 100% of their carbon from the plant and this increase in carbon flow to the roots, estimated up to 20% of plant photosynthates, translates to a huge amount of carbon worldwide.

AM symbiosis is often associated with improved plant growth. This enhanced growth has been attributed to nutritional and non-nutritional effects of AM fungi. It has been reported to benefit plants by increasing the uptake of nutrients such as P, Zn, Cu, and nitrogen. The non nutritional effects of AM would be due to increased tolerance to saline conditions, improved water relations, increased survival rate of transplanted seedlings, control of root diseases and increased soil aggregation by the external hyphal net work (Dodd and Thompson, 1994). The Mycorrhizal fungi are also known to produce wide array of plant growth promoting substances (IAA, IBA, GA). On perusal of the recent works carried out in the Tamil Nadu Agricultural University indicated that plants with coinoculation of AM fungi with other synergistic microbes like PGPR, mainly improved the growth and recorded increased yield of crop plants like rice, groundnut, maize mulberry, banana, pepper nutmug, clove, cardamom, papaya, trifoliate orange, onion, tapioca, sweet potato, tomato, moringa, gourds, and other flower crops (Fig 1). Thus, the AM symbiosis play a significant role in ensuring increased plant growth and yield by their synergistic interactions with different groups of microbes like N_2 fixers, P mobilizers, biocontrol agents, etc.

Positive interactions of AM fungi with soil microorganisms

Plant development may be improved by the combination of mycorrhizal fungi and rhizosphere microorganisms acting incoordination at the root soil interface (Lindermann, 1992) AM fungi are the key components of soil microbiota and the regulation of mycorrhizal formation is influenced by soil microorganisms (Azcon Aguilar and Barea, 1992).

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Interactions between mycorrhizal fungi and soil microorganisms involve nutrient cycling with impact on plant growth and nutrition. However manipulation of these beneficial combinations of microorganisms depends on the understanding of the ecosystem in order to apply a suitable selection of these microorganisms. Microbial compounds produced by soil microorganisms that increase root cell permeability, such as plant hormones, are involved in the formation of the symbiosis. As a consequence of mycorrhizal formation changes in the nutritional and physiological plant status occur. The root exudates modified by the mycorrhizal condition, in turn affect soil microbial populations. The term mycorrhizoaphere describes the influence of mycorrhiza on the rhizosphere zone.

positive response on improving the growth of several plant species. VAM fungal inoculation was found to stimulate the inflow of sugar from shoot to root in soybean which might alter the carbon availability for any bacteria in the endorhizosphere (*Volpin and Kapulnick*, 1994). In general ,the combined inoculation of AM and *Azospirillum* increased the growth, nutrient uptake of plant nutrients, dry weight and yield on wide variety of agricultural and Horticultural crops when compared to individual inoculation under field conditions. The positive interaction of both could be due to direct effect on host physiology. Selection of AM fungi strains for the improvement of crop yields and diazotrophs efficiency should consider inter symbiont compatability in addition to host plant compatibility.

Table 1. Growth performance of *Eucalyptus tereticornis* in bauxite mine spoils (24 months after plantation)

Treatment	Height (cm)	Collar diameter (mm)	No.of Branches/plant	Survival (%)
Control	55.4 a	11.19 a	10.8 a	40
VAM	12.5 b	20.65 b	18.3 b	90
PSB	114 b	17.46 b	17.5b	90
Azo	123.4 b	21.0 b	15.1 b	91
VAM + PSB+ Azo	135.7 c	20.13 b	15.0b	95

Where, VAM – Vesicular Arbuscular Mycorrhizal fungi; PSB – Phosphate Solubilising Bacteria; Azo – *Azotobacter*

Table 2. Influence of AM fungi and PGPR on *Tectona grandis*

Treatments	Growth parameters					
	Plant height (cm)	Stem girth (mm)	Biomass		Bio volume Index	Quantiy Index
			Stem	Total		
Control	9.33^{g}	3.51^{f}	9.71^{e}	21.72^{f}	111.94	1.67
Glomus leptotichum	18.33^{bc}	5.71^{c}	12.95^{cd}	26.83^{de}	410.43	1.75
Bacillus coagulans	11.50^{fg}	4.36^{df}	9.81^{e}	22.64^{f}	318.61	1.71
Trichoderma harzianum	16.25^{cde}	4.75^{cd}	11.79^{de}	24.43^{e}	366.64	1.74
Azotobacter chroococcum	14.58^{cde}	5.28^{cd}	11.66^{de}	25.24^{e}	406.46	1.75
G. leptotichum + B. coagulans+ T. harzianum	19.17^{be}	5.78^{c}	14.79^{bc}	33.32^{b}	597.63	2.10
G. leptotichum + B. coagulans+ A. chroococcum	20.08^{b}	5.76^{c}	18.13^{a}	38.83^{a}	666.20	1.83
G. leptotichum +T. harzianum+ A. chroococcum	28.00^{a}	7.08^{a}	19.96^{a}	40.64^{a}	1403.53	2.18

Note: Means followed by the same letter in the column do not differ significantly at p= 0.05

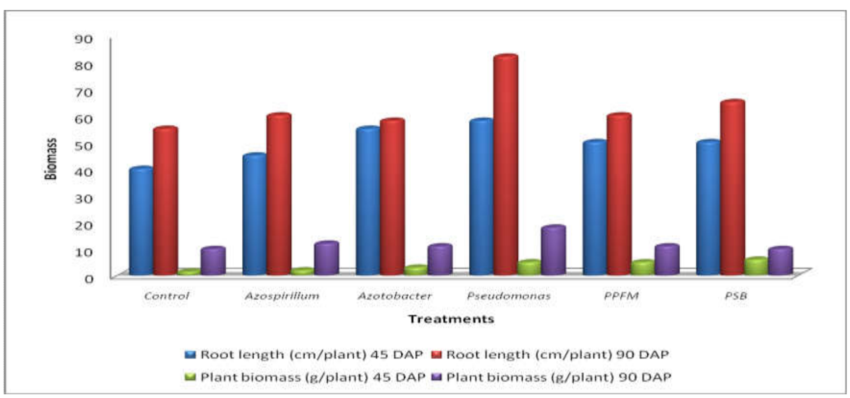

Fig.1. Influence of AM fungi and PGPR inoculants on growth of tomato

Interaction of AM with associative diazotrophs

VAM and rhizosphere microorganisms can influence their mutual development which might result in a symbiotic interaction. Very little information is available about the interaction between an associative diazotroph and AM fungi .Combined inoculation of *Azospirillum* and AM fungi studies at Tamil Nadu Agricultural University indicated that there is a

Interaction of AM fungi with PGPR organisms

a) *Pseudomonas fluorescens*

There are interesting indications that bacteria which are most abundant in soils interact with AM and play an important role in root. These so called mycorrhiza helper bacteria (MHB) enhance the extent of fungal colonization (Meyer and

Linderman, 1986) in clover. Dual inoculation of these two organisms has resulted in increased mycorrhizal colonization which may be due to increased spore germination (Mayo *et al*., 1986). There is some evidence that MHB produce cell wall degrading enzymes that might soften root cell walls and thus make it easier for VAM to penetrate the root or due to growth promoting substances the cell wall plasticity may get increased (Bagyaraj, 1989). Inoculation of sunflower with *Glomus fasciculatum, Pseudomonas striata* and *Azotobacter* resulted an increase of *Azotobacter* and *Phosphobacterial* population with increasing P concentration in host plants. Dual inoculation with both VAM and *Pseudomonas* had increase in population than when inoculated individually (Shivcharan, 1992). Kothari and Singh (1996), studied the response of *Citronella java* (*Cymbopogan winterianus jowitt*) to VA-mycorrhizal inoculation in a P-deficient sandy soil and found that inoculation of *Glomus intraradices* substantially increased root and shoot biomass, root length and nutrient uptake compared to inoculation with rhizosphere microorganisms in soil. Even growth performance significantly increased in case of *Eucalyptus* in bauxite mine spoils when inoculated with AM and some of the PGPR's (Table 1). Combined inoculation of *G. fasciculatum* MGF 3 and *Pseudomonas* sp. MPs 5 recorded 19.9 per cent increase in shoot weight, 16.3 per cent increase in leaf weight of mulberry (MR 2) over inoculation of *G. fasciculatum* alone (Kumutha, 2001). Dual inoculation of *Glomus mosseae* with *P.fluorescens* showed maximum VAM colonization per cent and spore count in *Coleus forskohlii* while experimenting biological control of root rot in this crop. (Boby and Bagyaraj, 2003). Bijily and Anil Kumar (2006) carried out a nursery trial consisting of combinations of bioinoculants viz., *Azospirillum* (Az), Fluorescent Pseudomonads (FP) and Arbuscular Mycorrhizal fungi (AM), for their effect of influencing higher productivity in long pepper (*Piper longum*) and reported increase in leaf number due to dual inoculation of FP + AMF and combined inoculation of AZ+FP+AMP. These bioinoculants along with vermicompost @ 6.25 t ha^{-1} and NPK @ 30:30:60 kg ha^{-1} $year^{-1}$ was found to enhance total fresh and dry spike yield and total alkaloid production in long pepper.

Kamal Prasad (2006) conducted a pot culture experiment to study the effect of dual inoculation *Glomus fasciculatum* and *Pseudomonas striata* on the medicinal crop *Azadirachta indica* and reported that there was a significant increase in root infection, spore population, stem height, number of leaves and total dry weight when compared to single inoculations as well as control. A maximum of 136.54%, 92.98% and 79.62% increase in stem height was registered due to dual inoculation of *G. fasciculatum* and *P. striata* and single inoculation of *G. fasciculatum* as well as *P. striata* at 90 days after planting respectively, and there was also increase in NPK uptake due to dual inoculation. Balakumbahan *et al*. (2006) studied on the effect of biofertilizers and inorganic nutrients on alkaloid content in Keelanelli (*Phyllanthus amarus*) and reported highest yield of the alkaloids, phyllanthin and hypophyllanthin due to application of Azosphos and VAM along with 75 kg Nha^{-1} + 37.5 kg P ha^{-1}. Even several notable evidence of the influence of AM with combined inoculation with PGPR was recorded in *Tectona grandis* (Table 2).

Sailo and Bagyaraj (2006) conducted a glass house investigation to study the influence of the AM fungus, (*Glomus bagyaraji*) and plant growth promoting rhizomicroorganisms (PGPRs), *Pseudomonas fluorescens, Piriformospora indica* and *Trichoderma harzianum*, alone and in combination, on the growth and nutrition of *Coleus forskohlii* and resulted that individual inoculation of these organisms significantly enchanced plant biomass, especially that root containing the ingredient, but biomass was significantly greater with inoculation of *G. bagyaraji* than with inoculation PGPRs. Dual inoculation of *G. bagyaraji* plus *Trichoderma harzianum* further significantly enhanced root biomass (Plate 1), plant height, number of branches, root length, plant P content, per cent mycorhizal colonization , spore numbers in the rhizosphere and forskohlin concentration compared with all other treatments.

Plate 1. Enhanced root biomass by dual inoculation of *Glomus bagyaraji* plus *Trichoderma harzianum* in Maize

b) *Trichoderma viride*

Most of the studies suggest that mycorrhiza decrease the severity of disease caused by root pathogenic fungi, bacteria and nematodes. Reduction in the severity of disease has been attributed to morphological alteration caused by mycorrhiza on the host plant like thickening of the cell wall, stronger vascular bundles, less giant cells etc. or because of physiological changes in the host like increased concentration of phosphorus, phenols, sulphur containing amino acid etc. (Bagyaraj, 1989). Calvet *et al*. (1990) reported that there was a significant increase in spore numbers of *G. mosseae* when inoculated with *T. viride* and later he reported that it was due to production of volatile compounds by *T. viride*. This type of synergism between *T.viride* and *G. mosseae* has been reported in case of marigold (Calvet *et al*., 1993) and in *Citrus reshni* by Camprubi *et al.* (1995). Molecular and biochemical analyses during AM colonization of the root cortex showed that elicitation of certain defence- related molecules does occur (Gianinazzi *et al.,* 1996). Boby and Bagyaraj (2003) conducted a field study for root rot control in *Coleus forskohlii* and reported that inoculation of *T. viride* with *Glomus mosseae* gave the best results with very less disease severity index of 33.28 per cent. In addition, they also showed a maximum growth, tuber and root forskohlin concentration.

Zhipeng *et al*. (2005) conducted a glass house pot experiment to investigate the impact of inoculation of cucumber at the germination stage with *Glomus etunicatum* BEG168 on plant yield and incidence of *Fusarium oxysporum f.*sp *cucumerinum* and resulted that the AM fungus inoculation decreased the disease index due to *Fusarium oxysporum.* Also, the mycorrhizal seedlings had higher concentration of phosphorus as well as proline and polyphenol oxidase activity than the non mycorrhizal seedlings. These indicated that the mycorrhizal fungus may influence plant secondary metabolites and increase resistance to wilt disease in cucumber seedlings and may therefore have the potential as biocontrol agent. Borges *et al*. (2007) carried out a green house experiment to evaluate the effect of AM fungi on the incidence and effect of *Fusarium oxysporum* f. sp. *cubense* (FOC) on Maca variety of banana (*Musa sp.*) during its initial growth, for which plantlets of banana were inoculated with *Gigaspora margarita* and after 60 days, they were inoculated with *Fusarium oxysporum. G. margarita* was efficient for growth of banana plantlets since previous inoculation of AMF resulted in bioprotection to *Fusarium oxysporum* and reduced disease index.

Interaction of AM with other beneficial microbes

The interaction of VAM fungi with rhizobia, bradyrhizobia and frankia were reported to be beneficial in legume plants as well as in non legume tree crops. Dual inoculation enhanced nodulation, nodule mass, ARA, plant biomass, N, P and micro nutrients status. In contrast to pre symbiotic phase where the interaction has direct effect on fungus, during symbiosis the interaction between soil microorganism and VAM is more related to plant growth and nutrition. Once the plant is mycorrhized it can cope up with higher P demand for N_2 fixation.

Conclusion

To maximize the beneficial plant growth response it is to identify the best strains of microorganism, verify their compatibility and combined efficiency before using them in agricultural management and production systems. AM fungi are considered to be the potential candidate for the sustainable system of agriculture, horticulture and forestry. Hence, the synergistic interactions of AM with beneficial soil microbes can be better utilized in the future to promote stable returns from agricultural point of view by eliminating crop failure.

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