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DETECTION OF INCREASED ROOT COLONIZATION OF MYCORRHIZAL FUNGUS IN PRESENCE OF SOIL YEASTS BY PCR BASED METHOD

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INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are involved in plant nutrient uptake, protection of plants against soil borne pathogens and improvement of soil stability. The considerable impact of AM fungi on plant growth places management of these fungi in an integrated role in agriculture (Smith and Read, 1997). The inoculum potential of AM fungi in soil is a key factor in soil quality, but the composition of fungal populations should also be addressed as fungal strains may differ in their effect on the host plant (Graham et al., 1982; Jacobsen et al., 1992). Thus specific detection of AM fungi complements studies of the agroecology of these fungi.

The necessity of spores to determine the species has seriously hampered ecological studies of AM fungi. The production of spores is not always correlated with root colonization (Clapp *et al*., 1995; Marryweather and Fitter, 1998). When no spores are formed, the intra radial structures of AM fungi at best allow identification up to the level of family (Marryweather and Fitter, 1998). Further more, several newly characterized lineages do not stain at all with standard procedures (Redecker *et al*., 2000). Although, hyphae are the most physiologically active states of the fungal part of symbiosis, hyphal morphology is inadequate for identification at the taxonomic level needed in most studies. These techniques are difficult to be used when more than one or two fungi are present in the roots and thus incomplete for field studies. For these reasons, several PCR based detection methods have been developed in recent years and some have already been applied in field conditions (Clapp *et al*., 1995).

In the present study arbuscular mycorrhizal fungus *G. mosseae* was used with soil yeasts to study the changes in root colonization in presence of soil yeasts. Fresh roots were collected from the cowpea grown in unsterile as well as sterilized soils at the time of harvest and were washed thoroughly to remove all the adhering soil particles. Mycorrhizal root colonisation was determined by the grid line intersect method by Giovanetti and Mosse (1980).

For PCR, DNA was obtained by crushing 10-20 mg of roots in 500 µl of extraction buffer (10 mM of Tris HCl, pH 8.5) for 15 minutes by taking in eppendorf tube using micro pestle. The template was diluted by 100 folds using double distilled water. The processed samples were immediately used for PCR. The reaction mixture consisted of 5 µl of DNA template, 250 µM of each dNTPs, 3 mM $MgCl_2$ and one unit of Taq polymerase (Genei, Bangalore), and 0.5µM each of VANS1 (GTCTAGTATAATCGTTATACAGG) and NS21 (AATATACGCT ATTGGAGCTGG) primers in a 25 µl total reaction volume.

The amplification was performed in a PTC-100 MJ research thermal cycler with 40 cycles of amplification with the following programme; denaturation at 94^{O}C for one minute, annealing at 50^{O}C for 45 seconds, extension for 60 seconds at 72^{O}C and a final extension at 72^{O}C for ten minutes.

Samples were analyzed in 2% agarose gel stained with ethidium bromide and photographed on UV light transilluminator. Inoculation of *G. mosseae* significantly increased root colonization compared to the control. When *G. mosseae* was inoculated with soil yeasts, it further increased root colonization in sterilized and unsterile soil conditions. In unsterile soil, maximum percent root colonization was observed in treatment with *G. mosseae* + *Rhodotorula mucilaginosa* (77.7%) which was significantly different from all other soil yeasts and *G. mosseae* treatment combinations, while *G. mosseae* + *Saccharomyces cerevisiae* (81%) resulted in maximum percent mycorrhizal root colonization in sterilised soil. *G. mosseae* alone treated plants

Table - 1: Colonisation by *G.mosseae* and PCR amplification results for colonised roots of cowpea plants

Treatments	Unsterile soil		Sterilized soil	
	Root colonisation (%)	Presence of PCR product	Root colonisation (%)	Presence of PCR product
Uninoculated control	40.0^{e}	+	0.0 ^{f}	-
G. mosseae (Gm)	52.0^{d}	+	66.0^{e}	+
Gm + *R. mucilaginosa*	77.7^{a}	+	72.7^{c}	+
Gm + *M. pulcherrima*	68.0^{c}	+	70.7^{cd}	+
Gm + *T cutaneum* var. *cutaneum*	70.3^{bc}	+	69.7^{d}	+
Gm + *S. cerevisiae*	72.7^{b}	+	81.0^{a}	+
Gm + *C. laurentii*	69.0^{c}	+	78.0^{b}	+
Gm + *D. occidentalis* var. *occidentalis*	69.7^{c}	+	69.0^{d}	+

had significantly higher per cent root colonization (52%) than uninoculated control (40). In sterilized soil, *G. mosseae* alone treated plants had significantly higher root colonization (66%) compared to uninoculated control which showed zero percent.

In this study the 550 bp rDNA amplification product characteristic of AM fungi was detected in all the treatments with *Glomus mosseae* and its combination with soil yeasts while uninoculated control plants did not show the amplification product. But in unsterile soil, 550 bp amplicon was noticed in all the treatments including uninoculated control plants. Simon *et al*. (1992) reported a portion of small subunit of rRNA gene (r DNA) specific for AM fungi could be amplified when a taxon specific primer (VANSI) is paired with universal primer (NS21). The 550 bp product was never detected from uninoculated, non-colonized control plants. None of the DNA template samples used for PCR in this study were purified and all the material had been exposed to normal glass house environment. Di Bonito *et al.* (1993) reported that AM fungi could be detected in the roots in the first few weeks of growth when the colonization was at least 30%, not the time when plants are at harvest, crucial for detection. This is evident from the amplification of a 550 bp in all the treatments including control in unsterile soil, which had a root colonization percent of 40%.

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