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International Journal of Current Research in Life Sciences
Vol. 07, No. 08, pp.2609-2612, August, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

CHARACTERIZATION OF SALINE TOLERANT STRAINS OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ON THE BASIS OF ISOZYME PATTERN USING POLY ACRYLAMIDE GEL ELECTROPHORESIS

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Received 04th June, 2018; Accepted 17th July, 2018; Published 30th August, 2018

ABSTRACT

Eight different saline tolerant strains of Arbuscular Mycorrhizal Fungi (AMF) were recovered from rhizosphere soil of plants growing in coastal saline soils in the West Coast of Kerala, India. They were characterized by subjecting spore extracts to polyacrylamide gel electrophoresis and selective enzyme staining. The activity of the Malate dehydrogenase (MDH) was measured after separation of the fungal enzymes on polyacrylamide gels. The banding patterns of malate dehydrogenase in these isolates were studied to identify the intrageneric variation in the activity of enzymes. The results showed only minor variations in the activity of the enzyme in all AMF species. Four distinct zones of MDH activity were detected on the gels based on the patterns of electrophoresis variations among the eight species of AM fungi. The study revealed that isoenzymic studies are useful for the differentiation and identification of AMF genera and species.

Key words: AMF, Rhizosphere, Poly acrylamide, Electrophoresis, Malate dehydrogenase.

Citation: Dr. Karthikeyan, C. "Characterization of saline tolerant strains of arbuscular mycorrhizal fungi (amf) on the basis of isozyme pattern using poly acrylamide gel electrophoresis" *International Journal of Current Research in Life Sciences*, 7, (08), 2609-2612.

INTRODUCTION

Arbuscular Mycorrhizal (AM) fungi are key microorganisms of the soil - plant system that are fundamental for soil fertility and plant nutrition (Smith and Read, 1997). They live in symbiosis with the roots of most plant species and produce spores in the soil, which are able to germinate in the absence of host - derived signals but unable to complete their life cycle without establishing a functional symbiosis with a host plant (Giovannetti, 2000). AM fungi are usually classified on the basis of the morphology of their resting spores. The characters which have been proposed as an aid to identification of this group of fungi are anatomical differences shown by the hyphae when growing within the host tissues (Abott, 1982), form of the vehicles borne on external mycelium (Hall, 1984), immunological relationships between different fungi (Aldwell *et al.,* 1983) and mobility of specific enzymes during polyacrylamide gel electrophoresis (Hepper *et al.,* 1986, Sen and Hepper, 1986). The polyacrylamide gel electrophoresis method has been used to identify and quantify endophytes within roots in a competition experiment (Hepper *et al.,* 1986) using a grading system, but it is essential that the relationship between the intensity of the staining reaction on the gel and the amount of fungal material in the root is known.

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Enzyme electrophoretic mobility is one of the methods which can be used to distinguish between fungi at the species level. The mobility of proteins of specific enzymes during electrophoresis has been used to characterize many organisms including fungi (Garber, 1973). Many species of fungi exhibit variation in enzyme banding patterns both in terms of total buffer soluble proteins (Seviour *et al.,* 1985) and specific enzymes (Alfenas *et al.,* 1984: Maghrabi and Kish, 1985 a & b) but it is essential for identification that there should be similarities between isolates of the same species (Hepper *et al.,* 1988). The application of the electrophoretic technique in mycorrhizal studies enabled the detection of fungi and plant specific acid and alkaline phosphatase isozyme within AM roots (Gianinazzi – pearson and Gianinazzi, 1976). Isozyme techniques have featured in taxonomic and population genetic studies of AM fungi. (Hepper *et al.,* 1988; Rosendhahl, 1989). The methodology has also been further extended to enable the identification of different fungal symbionts in endomycorrhizas (Rosendahl and Hepper, 1987) and ectomycorrhizas (Sen, 1990). The isozymic pattern of malate dehydrogenase (MDH) which is essentially an enzyme of the TCA cycle can be used for the identification and differentiation of many species / genera of AM fungi (Rosendahl, 1989: Sen and Hepper, 1986). In the present investigation, the identification and differentiation in the isozymic mobilities of different AM fungi species selected from the coastal saline soils of the west coast of Kerala was done.

MATERIALS AND METHODS

Spore collection: Spores of eight different AM fungi (*Acaulospora scrobiculata, Glomus aggregatum, G.deserticola, G.geosporum, G. microcarpam, G.margarita, Scutellospora heterogama and Sclerocystis pakistanika)* were isolated from the rhizopshere soil of plants (*Ipomoea pes-caprae and Phyla nodiflora*) growing in five different coastal saline soil localities each from 5 different districts of west coast of Kerala, India. Spores were extracted from rhizospheric soils of plants by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) to a mesh size of 100µm. Spores retained on sieves were flushed into petridishes and manually collected with a capillary pipette under a dissection microscope. Only intact, healthy spores were selected and placed in an Eppendorf tube. The spores were washed three times in sterile distilled water.

Sample Preparation: Before electrophoresis, spores were placed in 5µl of buffered sucrose (150 gl^{-1}) containing bromophenol blue tracking dye (20 mgl^{-1}). The buffer contained 20mM Tris HCl, 10mM $NaHCO_3$,10mM $MgCl_2$ 10mM Beta Mercaptoethanol and 0.1 mM EDTA, pH 8.0 (Bird *et al*., 1982) at 4 ^{0}C. The spores were crushed in the buffer using fine forceps and a further 5µl of buffered sucrose containing 0.6gl^{-1} Triton X-100 was added to the sample. Samples were prepared rapidly and maintained at 6 ^{0}C.

Electrophoresis: Samples were subjected to electrophoresis in vertical polyacrylamide gels (82 x 72 x 3mm). The stacking gel contained 3.75% acrylamide and 0.124 M Tris- HCl with pH 6.8 and the separating gel contained 7.5 % acrylamide and 0.375 M Tris- HCl with pH 8.8. The electrode buffer contained 3g Trisma base and 14.4g glycine per litre (pH 8.3). Each well was loaded wtih10µl of the sample. Electric current of 50V and 100 V was run through the gel for three hours. The gels were maintained in the cold room at 4^{0}C.

Enzyme Staining: The staining solution of MDH is a buffer, at optimum pH, containing artificial substrate, co – factor and dye. On incubation, coloured bands appeared in the gel at the zones of isozyme activity (Shankar and Varma, 1993). After electrophoresis, the gel was removed from the glass cassette and submerged into enzyme staining solution. The gel box was kept in the dark at 37^{0}C for one and a half hours. Dark blue bands appeared. The gel was photographed immediately after the staining. The banding patterns were recorded by measuring the migration from the gel interface and the staining intensity of each band of enzyme activity was recorded.

RESULTS

In the present study, electrophoretic patterns of spores of eight different species of dominant saline tolerant strains of AM fungi were analyzed by selective enzyme staining on Polyacrylamide Gel Electrophoresis (PAGE). The patterns of Malate dehydrogenase (MDH) were well resolved and reproducible, all AM fungi isolates showed four bands indicating the presence of isozymes with some variations in intensity and position. Four distinct zones of MDH activity were detected on the gels based on the patterns of electrophoretic variations among the eight saline tolerant strains of AM fungi (fig 1). Zone I was very slow moving and close to the cathode (point of origin). Zone II was fast moving compared to zone I and was located relatively closer towards the anode (running front) and zone III and IV moved ahead of zone II and were the fastest moving and located very close to the running front. The zymogram of eight AM fungi isolates showed four discrete zones of MDH activity (fig 2). All the four zones of MDH activity were different in their electrophoretic mobilities and intensity of protein staining. Four bands could be seen in MDH activity in all the seven mycorrhizal fungi, this indicates the presence of four major isoenzymes. But in the *Gigaspora margarita*, five bands were visible. Of the four zones of activity, maximum activity was observed in zone I in most of the isolates (four out of six isolates), except in *Glomus deserticola* and *Scutellospora heterogamma* where the activity of MDH in zone III was maximum. In each zone of MDH activity, one prominent intensely stained or two bands of differentially stained intensely stained or two bands of differentially stained intensity were observed. Intensely stained bands were observed in zone II of *G.aggregatum, G.geoporum and G.microcarpum* and zone III of G.*deserticola* and *Scutellospora heterogamma,* the intensely stained band was a fast moving one whereas the most faintly stained band was slow moving. But in *G. geosporum,* the intensely stained band was slow moving and near to the cathode and the fast moving band was faint.

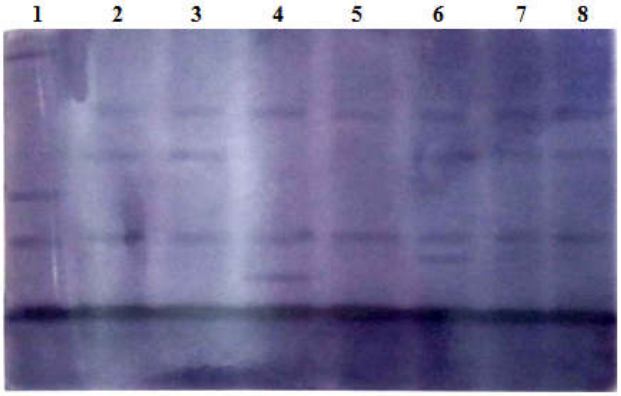

Fig.1. Isozyme (Malate dehydrogenase) pattern of eight different saline tolerant strains of AM fungi

Lane 1: *Glomus aggregatum*Lane 5: *Acaulospora scrobiculata*
Lane 2: *Glomus geosporum*Lane 6: *Gigaspora margarita*
Lane 3: *Glomus deserticola*Lane 7: *Scutellospora heterogama*
Lane 4: *Glomus microcarpum*Lane 8: *Sclerocystis pakistanika*

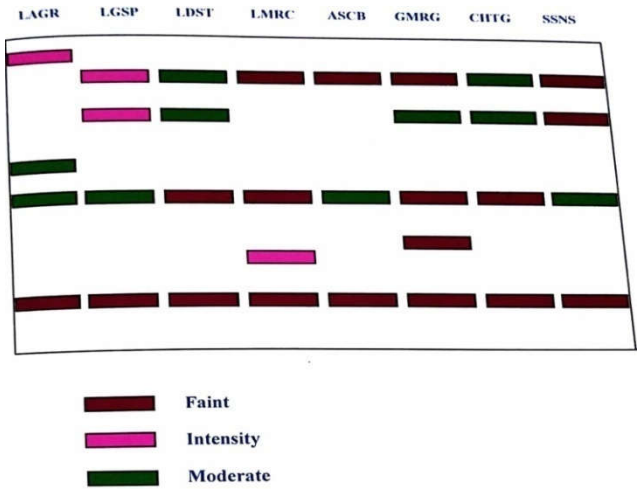

Fig.2. Zymogram depicting Isozyme (Malate dehydrogenase) pattern of eight different saline tolerant strains of AM fungi

LAGR: *Glomus aggregatum* ASCB: *Acaulospora scrobiculata*
LGSP: *Glomus geosporum* GMRG: *Gigaspora margarita*
LDST: *Glomus deserticola* CHTG: *Scutellospora heterogama*
LMRC: *Glomus microcarpum* SSNS: *Sclerocystis pakistanika*

DISUCSSION

Isozyme technique has featured in taxonomic and population genetic studies of AM fungi (Rosendahl, 1989: Shankar and Varma, 1993). The methodology has also been extended to enable the identification of different AM fungi symbionts (Rosendahl and Hepper, 1987). The isozymic patterns of Malate dehydrogenase (MDH) which is essentially an enzyme of the TCA cycle can be used for the identification and differentiation of many species of genera of AM fungi (Hepper *et al.,* 1988: Sen and Hepper, 1986; Shankar and Varma, 1993). *G. deserticola, G. fasciculatum* and *S. gilmorei* spores recovered from arid and semiarid regions have shown almost the same banding pattern of MDH in different zones of activity. But some additional allozymes were observed in arid region isolates (Shankar and Varma, 1993). In this study of eight saline strains of AM fungi, spores of four species of chlamydosporic forms of *Glomus* viz., *G. deserticola, G. aggregatum, G. geosporum* and *G. microcarpum,* one sporocarpic species of *Sclerocystis pakistanika* and three azygosporic forms *Acaulospora scrobiculata, Gigaspora margarita* and *Scutellospora heterogama* recovered from coastal saline soils have shown the same banding pattern of MDH in different zones of activity. In *G.desertiorla* and *G.microcarpum* zone II showed one allozyme while it was absent in other isolates of azygosporic forms.

Similar results were observed with *G.geosporum* and *G.aggregatum* in zone I, indicating that the mobility of these enzymes (which is based on the molecular weight and charge) was not very much influenced by the environmental or physiological conditions. It was further observed that saline isolates of different species had more isozymes, which may be a manifestation of adaptation to the stress conditions of the saline habitat. Evidences obtained so far suggest that the degree of variability observed between different isolates of a particular species of AM fungi in terms of number of isozymes detected was not greater than that observed in other fungi. Similar results were also observed in semi arid and AM fungal isolates of *G. deserticola, G. fasiculatum* and *S. gilmorei* (Shankar and Varma, 1993). In the present study, the AMF species exhibited considerable intrageneric variations in the banding patterns of MDH. The fungi which had identical spore morphology were identifiable as different species through specific enzyme staining that are highly sensitive. Only a small number of spores are required (Comparable with the number (20 -50) suggested by Trappe and Scheck (1982) for characterization by microscopical observation). For taxonomic studies, these techniques also provide a sensitive method for investigating the biochemical activities of the resting spores of AM fungi. They have already provided direct evidence of the presence of MDH activity in most of the spore types.

Conclusion: Four distinct Zones of MDH activity were detected on the gels, based on the pattern of electrophoretic variations among the eight species of AM fungi. In each zone of MDH activity, one prominent intensely stained or two bands of differentially stained intensity were observed. Intensely stained bands were observed in zone II of *G.aggregatum,, G.microcarpum, A.scrobiculata* and *S.pakistanika* and in Zone III of *G.deserticola ,G.margarita* and *S.heterogama*.

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