VARIATION IN THE SPORE DENSITY AND PERCENTAGE OF ROOT LENGTH OF TREE SPECIES COLONISED BY ARBUSCULAR MYCORRHIZAL FUNGIAT A REHABILITATED WATERLOGGED SITE

Anupama Gaur, Mahaveer P. Sharma, Alok Adholeya*

Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi 110 003, India

&c

Shashi Chauhan

Center for Studies in Microbiology, Jiwaji University, Gwalior 474 011, Madhya Pradesh, India

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GAUR, A., SHARMA, M.P., ADHOLEYA, A. & CHAUHAN, S. 1998. Variation in the spore density and percentage of root length of tree species colonised by arbnscular mycorrhizal fungi at a rehabilitated waterlogged site. A study was carried out at a rehabilitated locality in Haryana, north India, where waterlogging was a usual phenomenon that extended for more than nine months annually. Arbuscular mycorrhizal (AM) distribution in all the plots represented by various tree species, viz. Terminalia arjuna, Syzygium cuminii, Populus euphratica and naturally grown Typha elephantina was found to be significantly different. Distribution profile showed the dominance of the two genera, viz. Glomus and Gigaspora. Glomus was dominant in the T. arjuna plot while Gigaspora was abundant in the P. euphratica plot. Glomus showed positive correlation to available soil P (r = 0.74, p = 0.05) while Gigaspora showed positive correlation to organic matter content (r = 0.94, p = 0.05). Both the genera also predominated at the naturally grown T. elephantina plot.

Key words: AM - distribution - wasteland - Populus euphratica - Terminalia arjuna -Syzygium cuminii - Typha elephantina

GAUR, A., SHARMA, M.P., ADHOLEYA, A. & CHAUHAN, S. 1998. Perubahan dalam ketumpatan spora dan peratus panjang akar spesies pokok yang dijajah oleh kulat mikoriza arbuskular di tapak tanah air bertakung yang dipulihkan. Kajian dijalankan di Haryana, utara India di kawasan pemulihan tanah yang airnya bertakung dan merupakan kejadian biasa yang berlaku berlanjutan sehingga lebih sembilan bulan

^{*}Author for correspondence.

setiap tahun. Taburan mikoriza arbuskular di semua petak yang diwakili oleh berbagai spesies iaitu *Terminalia arjuna, Syzygium cuminii, Populus euphratica* dan *Typha elephantina* yang tumbuh semula jadi didapati berbeza dengan bererti. Bentuk taburan menunjukkan keunggulan dua genera iaitu *Glomus* dan *Gigaspora*. *Glomus* didapati unggul dalam petak *T. arjuna* manakala *Gigaspora* pula didapati berlebihan di petak *P. euphratica. Glomus* menunjukkan korelasi positif terhadap tanah tersedia P (r=0.74, p= 0.05) manakala *Gigaspora* menunjukkan korelasi positif terhadap tanah organik (r=0.94, p=0.05). Kedua-dua genera juga praunggul di petak *T. elephantina* yang tumbuh semula jadi.

Introduction

Waterlogged areas occupy an area of about 1 million hectares in several states of India. Punjab, Haryana and Uttar Pradesh are the worst affected states (Ram Prasad 1987). Vast expenses of wasteland cannot be left unutilised. Remedial measures employed to curtail waterlogging would be to utilise the surplus water through afforestation by planting suitable trees such as *Eucalyptus saligna*, *Dalbergia sissoo*, *Terminalia arjuna*, *Syzygium cuminii*, *Populus euphratica*, etc.

Arbuscular mycorrhiza (AM) appear to have a significant role in ecosystem function (Baylis 1967), but very little synecological research has been conducted, particularly on semi-arid or arid lands (Trappe 1981). Natural disturbances represent an added abiotic stress which results in soil disturbance and erosion (Naveh 1987). To reduce these negative effects and unfavourable environmental conditions, the soil stabilising (Bethlenfalvay & Newton 1991) and plant growth promoting (Comprubi et al. 1990) effects of arbuscular mycorrhizal symbiosis can be decisive for the successful establishment of revegetation strategies (Jasper 1994). However, when the top soil is removed, the indigenous population of mycorrhizal fungi is lowered and altered (Abbott & Gazey 1994). Although it is now widely accepted that AM fungi vary considerably in their symbiotic effectiveness according to host and soil conditions, the ways in which changes in physical and chemical properties of soil due to land reclamation influence the ecology and symbiotic behavior of arbuscular mycorrhizal fungi are poorly understood. Thus, the aim of the present study was to investigate the changes in the distribution pattern of the dominant genera (Glomus and Gigaspora) over a range of soil nutrients to different host species on a waterlogged site.

Material and methods

Study site

The study was conducted at the field research station of the Tata Energy Research Institute on the Aravalli hills, in the state of Haryana, India. The site (77° 12"E, 28° 35"N; 255 m above mean sea-level) receives an annual rainfall of 500 mm. The climate is typically subtropical. The soil is coarse, silty, mixed hyperthermic Typic Haplustalf derived from allium from mixed calcareous parent material and having argillic horizon at a depth of 58 to 168 cm. The locality, being low-lying had experienced heavy waterlogging in the rainy season and was dominated by *Typha elephantina* as the natural vegetation. Subsequently, waterlogging has been reduced with gradual rehabilitation of the locality with different tree species. The locality was divided into four different plots depending on the type of tree species planted in each plot. Naturally grown *T. elephantina* was maintained on Plot 1, while Plot 2 was planted with *Populus euphratica*, Plot 3 with *Terminalia arjuna* and Plot 4 with *Syzygium cuminii*. (Plots 2, 3 and 4 were planted in 1990.) In addition to the planted tree species, all the plots had a scanty cover of shallow rooted annual weeds, viz. *Argemone mexicana, Parthenium* sp. and *Cynodon* sp. A total of 25 trees were present each in Plots 2, 3 and 4 and Plot 1 was densely covered with *T. elephantina*.

Sample collection and processing for spore count and root colonisation

Soil samples were randomly procured in May 1995 from all the four plots with the help of soil augur (0-45 cms.) so as to represent the complete plot. Ten replicates from each plot were analysed. Root samples were collected from the plant rhizosphere. In order to collect finer roots detached due to soil disturbances during sampling, the rhizospheric soil was sieved using 20 BSS mesh to obtain whole root biomass. The procured root samples were homogenised thoroughly. The percentage of arbuscular-mycorrhizal colonised root length was determined by the method given by Biermann and Linderman (1981) after cleaning and staining (Philips & Hayman 1970).

Spores of AM fungi were extracted from the soil by the wet sieving and decanting method according to Gerdemann and Nicholson (1963). Total spore numbers of mycorrhizal fungi in the soil samples were estimated by the method of Gaur and Adholeya (1993). The isolated spores were picked up using stereo zoom (Leica 2000) and were mounted in polyvinyl lactoglycerol (PVLG) to make permanent slides and identification based on morphology, hyphal attachment, colour, size and wall layers using the identification manual for AM fungi by Morton and Benny (1990). Measurements were done using image analysis software (Quantimet 500+ Leica, Cambridge).

About 20-25 spores of the type that was most abundant in each soil sample were picked up to raise pure cultures using single spores by Funnel technique (Schenk 1988). The isolates were deposited in the Centre for Mycorrhizal Culture Collection (CMCC, TERI, New Delhi, India).

Chemical analysis

The soil samples collected were processed for chemical analysis to see the effect different tree species has on edaphic properties. Soil pH and electrical conductivity were measured (in a 1:2.5 soil to water ratio) using digital pH and EC

meter. Available phosphorus in soil samples was determined by extraction with sodium bicarbonate for 30 min (Olsen *et al.* 1954). Organic matter was estimated colorimetrically (Metson 1956).

Statistical analysis

The recorded data on spore density, mycorrhizal colonisation percentage (MCP) and soil nutrients from each plot were analysed using one way analysis of variance (ANOVA), least significant difference (LSD) with the Duncan's multiple range test (DMRT) at 5% significant level. The correlation coefficients (of all the soil samples) of the type of spore present with available phosphorus and organic matter were drawn using Costat Stastical Software (Cohort, PO Box 1149, Berkley, CA 94701, USA).

Results

In the present study, the percentage root length colonised was found significantly different (p=0.05) for each of the plots studied. A maximum of 56% colonisation was recorded in the roots of *P. euphratica* (Plot 2) followed by 51% in *T. elephantina* (Plot 1), 41% in *Terminalia arjuna* (Plot 4) and the lowest of 12.5% was observed in *S. cuminii* (Plot 3).

Spore density was also found to differ among the various plots sampled. *Glomus* dominated at Plot 4 with a spore density of 5.9 spores g^{-1} , followed by Plot 3 having 5.6 spores g^{-1} , Plot 2 and Plot 1 with 3.98, 4.6 spores g^{-1} respectively (Figure 1). In contrast, the population of *Gigaspora* was found to be maximum at Plot 2 having a spore density of 11.8 spores g^{-1} followed by Plots 1, 3 and 4 having spore densities of 5.7, 4.5 and 3.5 spores g^{-1} respectively (Figure 1).

ANOVA worked out for various soil chemical parameters revealed that a great variability in terms of organic matter and available P existed among the various plantation plots. A significantly higher organic matter (8.6%) and available P (3.85 mg g⁻¹) was recorded in Plots 2 and 4 respectively (Table 1).

Locality (Plot)	рН	Electrical conductivity (dS m ⁻¹)	Organic matter (%)	Available P (µg g ⁻¹)	MCP (%)	Initial VAM profile (before plantation 1998) (spores g ⁻¹ soil)
1	7.54b	0.30a	5.86b	1.69c	51b	1.8a
2	7.54b	0.21c	8.66a	1.21d	56a	0.86b
3	7.54b	0.29b	3.70d	1.86b	41c	1.79a
4	7.57a	0.17d	4.74c	3.85a	12.5d	0.91b
LSD	0.021	0.001	0.262	0.219	0.859	0.220

Table 1. Soil characteristics in various plots of the study sites

*LSD = least significant difference;

MCP = mycorrhizal colonisation percentage.

Means followed by the same letter did not differ significantly (p = 0.05).

There was a distinct correlation (negative or positive) between the multiplication of the AM genera and soil nutrients (Figures 2 and 3). The increment of *Glomus* was significantly correlated (r = 0.74) to increasing available soil P levels in all the plots, the maximum being at 3.9 ppm available P, whereas *Gigaspora* had significantly positive correlation with organic matter content (r = 0.94).



Figure 1. Means of spore number (genera Glomus and Gigaspora) of arbuscular mycorrhizal fungi present at the study site. (Means followed by the same letter did not differ significantly. The bars represent standard deviation of 10 replicates.)

There was no significant difference found for pH in almost all the plots except Plot 4. Electrical conductivity was found to be significantly different in all the four plots at the time of observation. Initial total spore count was not significantly different between Plots 1 and 3, and Plots 2 and 4 (Table 1). All the four plots showed wide variations in the distribution of AM and extent of colonisation among the plant species used for rehabilitation. Though the earlier observation indicated dominance of *Glomus* at the site (unpublished data), the scenario has been changed with the change of soil nutrient status. The total spore count initially was comparatively lower (Table 1).



Figure 2. Relationship of available phosphorus ($\mu g g^1$) with the distribution of *Glomus* (y = 3.72 - 0.65x) and *Gigaspora* (y = 9.6 - 1.96x) in the rehabilitated sites. (The bars represent standard deviation of 10 replicates.)



Figure 3. Relationship of organic matter (%) with the distribution of *Glomus* (y=6.42 - 0.46x) and *Gigaspora* (y = 1.89 + 2.51x) in the rehabilitated sites. (The bars represent standard deviation of 10 replicates.)

Discussion

Highest spore population of *Glomus* was obtained in Plot 4 dominated by *T. arjuna* with significantly higher available P (3.9 ppm). Though the distribution of AM fungi differs according to the prevailing edaphic conditions, the nutritional requirements of various genera are still not clear. Our observation that *Glomus* was prevailing in the soil with high available P is in agreement with various reports (Hayman & Stovold 1979, Jensen & Jacobsen 1980, Young *et al.* 1985, Jeffries *et al.* 1988) that many species of *Glomus* seem to be well adapted to fertile soils with a high nutrient level. Puess and Gianinazzi-Pearson (1984) also observed that populations of *Glomus* are significantly influenced by phosphate levels. The positive correlation between the spore density of *Glomus* and the available soil P observed in the study was also in accordance with the observations of *Abbott* and Robson (1982) who reported an increase in spore population of *Glomus* with increase in available soil P. Moreover, *T. arjuna* dominating plot 4 has a very superficial root system making its dependence on arbuscular mycorrhizal fungi all the more important in such deficient subtropical soils (Mehrotra 1991).

On the other hand, the Gigaspora in this study was found to be correlated to increasing content of soil organic matter; a significantly higher Gigaspora population was recorded in Plot 2 (with *P. euphratica*) having the highest organic matter content (8.7%). Lodge (1989) has also reported the formation of AM association in *P. euphratica*. High organic matter in this plot can be ascribed to leaf tissue of *P. euphratica* which is soft and smaller than those of other tree species under study and is observed to disintegrate easily into the soil. A positive correlation between the frequency of AM fungi and the percentage of organic matter in the soil has often been proposed (Howard 1948, Hayman 1974, Harinikumar & Bagyaraj 1989). Moreover, Land and Schönbeck (1991) reported in their studies the dominance of Gigaspora in soils with organic matter content of 3.5%. A similar observation was recorded in our study where organic matter level up to 8.7% supported the abundance of Gigaspora. In contrast, Redhead (1977), Koske (1987) and Gemma *et al.* (1989) reported the abundance of Scutellospora, Acaulospora and Gigaspora in low nutrient or nutrient binding soils.

Soil disturbance (Moorman & Reeves 1979, Reeves et al. 1979, McGonigle et al. 1990) and plant community structure (Johnson et al. 1991, 1992) can also affect the composition of AM fungal communities. A change in host plant composition is likely to alter the activity and species composition of AM fungi as well. Also, Johnson et al. (1992) demonstrated that changes in plant composition resulted in concomitant changes in the composition of the AM fungal community. This is in accordance with our observation that initially waterlogged locality had very low population of AM (Table 1), but with gradual rehabilitation, different plant species resulted in changes in the composition of AM fungal communities.

AM inoculation had a positive effect on plant survival which is especially important in reforestation activities (Estuan *et al.* 1997). This makes mycorrhiza research in such disturbed subtropical areas a very important issue. Soils in such areas are deficient in phosphorus (Garg 1987) (as we can also see from the data) and phosphorus nutrition is the bottleneck in tropical forestry (Norani 1996). Thus, mycorrhizal fungi offer great potential in reclamation of such sites with inherent stress. All the tree species selected along with their associated symbionts helped in the removal of excess water from the soil surface. The fibrous and deep root systems may absorb water from soil layers below the surface, resulting in decrease of evaporation from soils and reduction of Na accumulation near the soil surface. Arbuscular mycorrhizas in turn increase waterflow via the fungal hyphae (Allen 1982), improve P nutrition (Fitter 1988) and have better plant water absorption and transpiration. The inoculation of plants for revegetation purpose poses a dual function, the most obvious being the direct benefits to the inoculated plants. The higher growth rate, especially under nutrient deficient tropical conditions, achieved by inoculation results in increased soil coverage that helps in soil protection against erosion. There are increasing reports that the diversity of glomalean fungal communities decreases when natural ecosystems are disturbed by human activities (Johnson & Pfleger 1992) thus causing selective pressure on arbuscular mycorrhizal fungal communities.

Further studies are needed to determine the factors responsible for distribution of arbuscular mycorrhizal fungi and to understand the mechanism of these factors in an ecosystem. In the present investigation, the dominance of the two genera was significant for the survival of the native vegetation in the studied ecosystem.

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