Tropical Ecology **44**(2): 253-256, 2003 ISSN 0564-3295

Vesicular-arbuscular mycorrhizal association in naturally revegetated coal mine spoil

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Key words: Association, coal mine spoil, VAM.

A major beneficial component of soil microbial community is mycorrhizal fungus, which contributes to plant growth and survival by reducing stresses through symbiosis (Sylvia & Williams 1992). There are several studies reporting the role of mycorrhizae in stressed habitats. The mycorrhizae are very common in disturbed areas which indicate their positive role in establishing and building the plant community. Also, the mycorrhizal associations are essential to the colonization of nutrient-deficient soil heaps left after mining. There are evidences that VAM determine the rate of succession in mined land (Dorr *et al.* 1984).

In India, only during the last decade the interest aroused on the role of VAM in rehabilitation of disturbed ecosystems (Kumar *et al.* 1999). However, because of various constrains work done in India is not enough to suggest a suitable mycorrhizal technology for revegetation of coal mine spoils. Therefore, the present work was done to assess the VAM association in plants colonizing and growing naturally on various mine dumps of Jayant.

The study was conducted at Jayant mining block of Northern Coal Fields Ltd., Singrauli which is situated in Madhya Pradesh. The area is situated between the lat. of 23°47′ – 24°12′ N and long. of 81°48′ – 82°52′ E and is at an elevation of 280 – 519 m above msl. The climate of the region is tropical monsoon and the year is divisible into a mild winter (November-February), a hot summer (April-June), and a warm rainy season (July-September), while the other months are transitory periods between these seasons. Three naturally revegetated sites viz. 6–yr old dump in east section, 9–yr old dump in west section and 12-yr old dump near view point were selected for the study.

Fine terminal feeder roots were collected during rainy season in 1995 from different places of the root system and were mixed together to get a composite sample. This procedure was followed for each plant species and the roots were collected in separate polythene bags. These root samples were brought to laboratory, and were preserved in small plastic vials with formalin-acetone-alcohol (FAA) in the ratio of 90:5:5 (v/v/v). From these preserved root samples sub-samples were drawn for studying the level of VAM colonization.

Chlorazol Black E in lactoglycerol was used to stain the roots that were cleared by heating in KOH (Phillips & Hayman 1970). The root samples stored in FAA were washed thoroughly with tap water and then they were transferred to autoclavable Eppendorf tubes with small pores at bottom to allow the chemical to pass through it. The tubes containing root samples were placed in 1000 ml beaker containing 10% KOH (w/v). A Petri plate was put over the beaker to keep the tubes submerged in the KOH solution. The beaker containing the specimen was placed in autoclave for 15 min at 1.03 x 10^5 N/m^2 (15 psi) pressure. After autoclaving, the KOH solution was poured off from the beaker and the tubes were rinsed with several changes of distilled water. The tubes were put in a beaker containing 1% HCl solution for 5 min to acidify the roots. The tubes containing roots were again rinsed several times with distilled water. After rinsing, the roots were again put in a beaker containing 0.03% Chlorazol Black E-lactoglycerol

staining solution and autoclaved again for 10 min at 1.03 x 10^5 N/m^2 (15 psi) for staining the roots. Heavily pigmented roots were bleached prior to staining with alkaline H_2O_2 (Phillips & Hayman 1970). The time required for bleaching varied with different root samples. The roots were then put between two slides, pressed gently, and were observed under a microscope for VAM colonization. The level of colonization in each root segment was measured by the method of Giovannetti & Mosse (1980).

A total of 79 plant species belonging to 30 families were surveyed for the mycorrhizal association growing naturally at coal mine dumps of various ages. Six families namely Poaceae (13 species), Fabaceae (7 species), Mimosaceae (5 species), Asteraceae (6 species), Moraceae (4 species) and Amaranthaceae (4 species) possessed most frequent VAM association. Out of seventy nine plants sampled, 5 plants namely *Blumea* sp., *Ipomoea hispida, Scoparia dulcis, Sida cordata*, and *Woodfordia fruticosa* did not possess any VAM association. Only one plant, viz. *Crotalaria albida* possessed very low level of association (less than 20%). Moderate level of association (20-49%) was recorded in 40 plant species. Some of the important species in this range were *Acacia auriculiformis, Acacia catechu, Albizia lebbeck, Albizia procera, Argemone mexicana, Cassia fistula, Casuarina equisetifolia, Cyperus rotundus, Dendrocalamus strictus, Diospyros melanoxylon, Eucalyptus hybrid, Euphorbia hirta, Ficus elastica, Heteropogon contortus, Melia azedarach, Pongamia glabra, Prosopis juliflora, Phyllanthus emblica* and *Shorea robusta*. High level of VAM colonization (50-69%) was recorded in 29 plants including *Acacia nilotica, Butea monosperma, Calotropis procera, Cynodon dactylon, Dalbergia sissoo, Ficus benghalensis, Greviella pteridofolia, Holarrhena antidysentrica, Holoptelia integrifolia, Madhuca indica, Morus alba, Ocimum basilicum, Pongamia pinnata, Syzygium cumini, Tamarindus indica, Terminalia bellirica, Tectona grandis* and *Terminalia arjuna*. Four plants namely *Azadirachta indica, Cassia siamea, Indigofera linifolia* and *Saccharum arundinaceum* possessed very high level (≥70%) of VAM colonization (Tables 1 & 2).

21 plants showed presence of VAM hyphae only in their roots where neither arbuscules nor vesicles were recorded. The important species possessing only mycorrhizal hyphae were *Acacia auriculiformis, Casuarina equisetifolia, Dendrocalamus strictus, Ficus elastica, Melia azedarach, Phyllanthus emblica, Pongamia glabra* and *Prosopis juliflora*. Twenty nine species contained hyphae along with arbuscules and vesicles; the important species were *Acacia nilotica, Butea monosperma, Cassia fistula, Grevillea pteridofolia, Holarrhena antidysentrica, Madhuca indica* and *Morus alba* while *Diospyros melanoxylon* possessed only arbuscules. Twenty three plant species contained hyphae along with vesicles in their roots. The important species in this category were *Acacia catechu Albizia lebbeck, Azadirachta indica, Dalbergia sissoo, Eucalyptus hybrid, Holoptelia integrifolia, Pongamia pinnata, Syzygium cumini, Tamarindus indica, Terminalia arjuna* and *T. bellirica* (Tables 1 & 2).

On the basis of the shape of the vesicles four categories of associations were identified in different plant species. 15 plant species possessed only one type of vesicles of which the important species were *A. catechu, C. procera, E. hybrid, T. grandis, T. bellirica,* while 28 plant species including *A. nilotica, A. procera, A. oocidentale, C. tora, F. religosa, G. pteridofolia, M. indica, P. pinnata, S. cumini, T. arjuna*, possessed two types of vesicles. In 9 plants viz. *A. lebbeck, A. indica, B. monosperma, C. siamea, C. rotundus, D. triflorum, F. benghalensis, I. linifolia, Zornia gibbosa* three types of vesicles were observed (Table 1).

During the study it was found that 94% of total plant species possessed VAM colonization. Out of these mycotrophic plants, only one plant possessed low level of VAM colonization. 51% plants possessed moderate level of VAM colonization and 42% plants possessed high to very high level of VAM colonization (Tables 1 & 2). Although it has been reported that VAM inoculum may be reduced due to disturbances (Brundrett 1991; Gould *et al.* 1996), a moderate to high level of mycorrhization observed in almost all the species in the present study reflects the importance of mycorrhizal association in plants colonizing the coal mine spoil. In most ecosystems a good appreciation of the overall importance of mycorrhiza can be obtained by combining floristic data (Takhtajan 1986) with mycorrhizal information.

The presence of arbuscules (Smith & Gianinazzi-Pearson 1988) is normally used to designate VAM association, but the presence of hyphae or vesicles alone has also been used as evidence for these associations. Arbuscules are ephemeral structures which may be absent if samples are col-

Table 1. Survey of mycotrophic and non-mycotrophic plant species grown at naturally revegetated coal mine dumps.

Host species / Family	Shape of vesicle	% VAM	Host species / Family	Shape of vesicle	% VAM
Acacia auriculiformis (Mimosaceae)*	–	20	*Ficus benghalensis* (Moraceae)	G, SG, E	65
A. catechu (Mimosaceae)	SG	43	*F. elastica* (Moraceae)*	–	30
A. nilotica (Mimosaceae)	G, SG	55	*F. religiosa* (Moraceae)	E, SG	20
Achyranthes aspera (Amaranthaceae)	SG, E	45	*Gmelina arborea* (Verbenaceae)*	–	23
Aegle marmelos (Rutaceae)	SG, G	40	*Grevillea pteridofolia* (Poaceae)	SG, G	50
Ageratum conyzoides (Asteraceae)*	–	50	*Hedyotis affinis* (Scrophulariaceae)*	–	35
Albizia lebbeck (Mimosaceae)	SG, G, E		*Heteropogan contortus* (Poaceae)*	–	30
A. procera (Mimosaceae)	SG, G	45	*Holoptelia integrifolia* (Ulmaceae)	SG, G	55
Alternanthera pungeus (Amaranthaceae)	SG	38	*Holarrhena antidysentrica* (Apocynaceae)	G, SG	65
A. sessilis (Amranthaceae)	G	42	*Indigofera linnifolia* (Fabaceae)	SG, G, IG	70
Anacardium occidentale (Anacardiaceae)	SG, E	20	*Ipomaea hispida* (Convolvulaceae)	–	–
Argemone mexicana (Papaveraceae)	G	35	*Leucas plukenetii* (Lamiaceae)	SG	42
Atylosia scarabaeoides (Fabaceae)	SG, G	20	*Merremia tridentata* (Convolvulaceae)	SG, G	56
Azadirachta indica (Meliaceae)	IG, SG, GE	70	*Madhuca indica* (Sapotaceae)	SG, G	56
Bidens biternata (Asteraceae)	SG, G	40	*Melia azedarach* (Meliaceae)*	–	30
Butea monosperma (Fabaceae)	IG, SG, G	50	*Morus alba* (Moraceae)	G, SG	60
Blumea sp. (Asteraceae)	–	–	*Ocimum basilicum* (Lamiaceae)	G, IG	55
Calotropis procera (Asclepadaceae)	SG	60	*Parthenium hysterophorus* (Asteraceae)*	–	20
Cassia fistula (Caesalpiniaceae)	SG	45	*Phyllanthus emblica* (Euphorbiaceae)*	–	40
C. siamea (Caesalpiniaceae)	E, SG, IG	73	*P. virgatus* (Euphorbiaceae)*	–	60
C. tora (Caesalpiniaceae)	SG, G	42	*Pongamia glabra* (Leguminoseae)*	–	40
Casuarina equisetifolia (Casuarinaceae)*	–	40	*P. pinnata* (Leguminoseae)	G, SG	60
Celosia argentea (Amaranthaceae)	SG, G	25	*Prosopis juliflora* (Leguminoseae)*	–	30
Clitoria sp. (Leguminoseae)*	–	30	*Saccharum arundinaceum* (Poaceae)	SG, G	70
Crotalaria albida (Fabaceae)*	–	10	*S. spontaneum* (Poaceae)	G	55
Cynodon dactylon (Poaceae)	G, SG	50	*Scoparia dulcis* (Scrophulariaceae)	–	–
Cyperus rotundus (Cyperaceae)	IG, SG, G	45	*Shorea robusta* (Dipterocarpaceae)*	–	30
Dactyloctenium aegyptium (Poaceae)	G	60	*Sida cordata* (Malvaceae)	–	–
Dalbergia sissoo (Fabaceae)	G, SG	58	*Sporobolus indicus* (Poaceae)*	–	20
Dendrocalamus strictus (Poaceae)*	–	30	*Syzygium cumini* (Myrtaceae)	SG, G	52
Desmodium triflorum (Fabaceae)	IG, SG, G	48	*Tamarindus indica* (Leguminoseae)	SG	50
Dichanthium annulatum (Poaceae)*	–	65	*Tectona grandis* (Verbenaceae)	G	60
Digitaria bicornis (Poaceae)*	–	57	*Terminalia arjuna* (Combretaceae)	SG, IG	60
Diospyros melanoxylon (Ebenaceae)**	–	40	*T. bellirica* (Combretaceae)	SG	50
Eclipta prostata (Asteraceae)	G, SG	52	*Tephrosia purpurea* (Poaceae)	SG, G	40
Eragrostis tenella (Poaceae)	G	40	*Tridax procumbens* (Asteraceae)	G, IG	45
E. uniloides (Poaceae)	SG, G	50	*Woodfordia fruticosa* (Lythraceae)	–	–
Eucalyptus hybrid (Myrtaceae)	G	40	*Zizyphus glaberrima* (Rhamnaceae)	SG	30
Euphorbia hirta (Euphorbiaceae)	SG, IG	48	*Zornia gibbosa* (Fabaceae)	SG, G, IG	58
Evolvulus alsinoides (Convolvulaceae)*	–	60			

Abbreviations: G, globose; SG, sub-globose; IG, iso-globose; E, ellipsoidal; *, hyphae alone; **, arbuscules with hyphae; –, absent

Table 2. Categorization of plant species having mycorrhizal association at naturally revegetating coal mine dumps.

Level of association		Type of VAM association*			
Categorization	Total Number	H+ AR+V	H	H + AR	H + V
Plant species	79	29	21	1	23
≥ 70% (very high)	4	2	0	0	2
50-69% (high)	29	14	5	0	10
20-49% (moderate)	40	13	15	1	11
< 20% (low)	1	0	1	0	0
No association	5	0	0	0	0

Abbreviations: *, Values represent number of plant species; H, hyphae alone; AR, arbuscule; V, vesicle

lected when roots are inactive, whereas vesicles are considered as storage organ produced in the older region of infection. Although 41% of the plants in the present study possessed arbuscules, the number of plants possessing vesicles was higher than the plants bearing arbuscules. The condition indicates that roots of majority of the plants colonized were mature.

All the plant species surveyed were growing naturally at the dump and majority of them possessed fairly good mycorrhizal association. This indicates that plant surveillance at revegetating coal mine spoil is affected by VAM infection. The reservoir of mycorrhizal inoculum on the mine dump in the present study seems to be the adjoining forest area from where the inoculum might have spread in this area through various agencies. The type of inoculum could be of any type, for example spores, hyphae, plant root debris, etc. Gould *et al.* (1996) have reported that mycorrhizal inoculum in spoil during the first year following dumping chiefly consists of spores and following the first spring after reclamation other forms of mycorrhizal inoculum are increased in a revegetating coal mine spoil. As the succession commences at disturbed site, there is an increase in VAM inoculum level and with the increase in plant root system more association arise.

Acknowledgements

The project was funded by the Ministry of Coal, Government of India, through Central Mine Planning and Design Institute (CMPDI) Ranchi. The authors owe their sincere thanks to Prof. J.S. Singh, Co-ordinator of the project whose endeavor and great interest managed not only to get the project launched but also led to its successful completion.

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