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Significance of Micro-organisms in Afforestation Programmes in Arid Zone

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Abstract: Trees play a significant role in sustaining the productivity in arid zones. But their establishment and growth in arid zone are poor because of harsh climate coupled with low fertility of light sandy soils. The effects of inoculating seeds of various trees with efficient strains of *Rhizobium* and inoculating soil with VAM-fungi, alone or in combination, appear promising. Further, dual inoculation of trees helps in mitigloneating the adverse effects of salinity. Nitrogen fixed in tree legumes was found to be transferred to the non-legumes when grown either in silvopastoral or agroforestry systems.

Key words: Afforestation, VAM-fungi, *Rhizobium*, N-fixation, arid zone.

In general, plant productivity in arid regions is very poor because of scanty and erratic rainfall, frequent droughts, poor fertility and low moisture holding capacity of soils. Due to the risk involved in agriculture, trees assume considerable importance as a source of fodder, firewood, timber, fencing material as wind break or shelter belts, etc. Nitrogen fixing trees (NFTs) are widely grown in agroforestry and silvipastoral systems for efficient use of sunlight and soil resources such as water and nutrients, and for sustainable productivity, even in bad rainfall years. Growth of native tree species in this region is very slow, particularly in early stages (Bray *et al.*, 1985). However, enhanced growth is possible through improved technology employing nitrogen fixing bacteria and vesicular-arbuscular mycorrhizal (VAM) fungi.

Biological Nitrogen Fixing (BNF) Systems

Since nitrogen is generally deficient in arid soils (0.03%), nitrogen supply to plants could be stepped up by growing NFTs for better growth and establishment of trees. The greatest potential for sustainable plant productivity lies with enhanced BNF. Nitrogen fixation occurs either symbiotically through legume - *Rhizobium* and non-legume-actinorhizal (*Casuarina-Frankia*) associations, or non-symbiotically in the rhizosphere of various trees. Inoculation of tree roots, with a suitable non-symbiotic N-fixing bacterium for higher N-fixation is a possibility, but research so far has mixed ~~success~~.

The most successful management of soil micro-organisms has been symbiotic nitrogen fixing bacterium. i.e., *Rhizobium*. Initially, the appropriate management strategy was inoculation with selected rhizobia suited for a particular legume species and soil. Studies conducted on a number of leguminous tree species have shown that they contribute 60-250 Kg N ha^{-1} to the soil, depending on the tree species, climate and soil type (Pokhriyal and Mathani, 1989). Effective nodulation of seedlings and ni-

Table 1. Nodulation and dry matter yield of 5 month old A. lebbek upon inoculation with different strains of Rhizobium

Treatment	Plant height (cm)	Biomass (g plant^{-1})	Nodule number (plant^{-1})	N_2-ase activity*
Control	25.3	7.8	4	2.8
Al_7	36.3	11.0	19	11.2
Al_8	40.3	15.0	44	17.9
Al_{14}	36.0	10.2	16	11.5
Al_{21}	42.8	16.8	44	18.5
LSD (P=0.05)	2.0	1.2	3.1	2.1

* µmoles C_2H_4 plant^{-1} h^{-1}.

trogen fixation is a pre-requisite for better establishment and growth after transplantation.

Extensive surveys were made to study the status of nodulation among trees (*Acacia*, *Prosopis*, *Albizia*, *Leucaena*, *Dichrostachys*, etc.) in arid zone. The root systems in these trees are deep but surface proliferation is common, depending upon the rainfall pattern, type of soil and presence or absence of a crop. In general, nodulation was rarely observed on the surface roots because of lack of specific rhizobia. But, we find that under agroforestry systems, when legume crops are grown in between trees, surface roots of leguminous trees develop nodules because of the presence of rhizobia of crop legume (mostly cowpea group of *Rhizobium*).

There is an urgent need to assess the performance of other tree legumes upon inoculation with introduced rhizobia, both under nursery and field conditions (outplantations). It was found that upon inoculation with different strains of *Rhizobium*, nodulation was significantly enhanced, besides improvement in plant height and biomass production (Table 1). The inoculated plants were more healthy and withstood stresses during transportation and outplantations. It is natural that when the nursery stock is healthy and sturdy, mortality rate is reduced and plants demonstrate better growth and development.

Among legume trees, BNF has been intensively studied in *Leucaena leucocephala*. Nitrogen fixation and biomass production of *L. leucocephala*, upon inoculation with efficient strains of *Rhizobium,* were better compared to uninoculated seedlings in the nursery (Sanginga *et al*., 1989a). It was observed that establishment and growth of *L. leucocephala* in the field was better when inoculated at the time of sowing and transplanting (Venkateswarlu *et al*., 1990). Further, nodulation, nitrogen fixation (N_2-ase activity), shoot dry weight and nitrogen uptake in transplanted seedlings were improved upon inoculation. In general, inoculation of seedlings at the time of transplantation is better compared to that inoculated at sowing, but inoculation at both the times results in maximum benefit to the plants.

An attempt was made to quantify the amount of nitrogen fixed in *L. diversifolia*

Table 2. Biomass production, nitrogen uptake and nitrogen fixation on L diversifolia as influenced by inoculation (Rao and Giller, 1993)

Treatment	Biomass (g pot^{-1})	N-uptake (mg pot^{-1})	^{15}N atom % excess	% Ndf Fix*
ISO-23	3.599	100.99	1.695	48.34
TAL-582	3.015	78.85	1.889	42.42
CIAT-1967	3.805	107.76	1.507	54.07
Control	2.285	58.81	3.281	-
LSD (P=0.05)	0.322	15.92	0.053	-

* %Ndf fix: per cent nitrogen derived from fixation.

by different strains of *Rhizobium* employing isotope dilution technique with ^{15}N (Rao and Giller, 1993). Inoculation significantly reduced the atom per cent ^{15}N excess compared with the reference plants, indicating thereby the higher N-uptake through fixation in inoculated plants (Table 2). ^{15}N enrichments among inoculated plants varied from one strain to another. About 42-54% of plant nitrogen in *L. diversifolia* was obtained from fixation, while Sanginga *et al.* (1989b) reported that only 36% of N in *L. leucocephala* was obtained from fixation.

Mycorrhiza

Evidence suggests that the mycorrhizal habit evolved as a survival mechanism and allows each to survive in the existing environment of low soil fertility, drought, disease and temperature extremes. Role of mycorrhizae in improving the quality of planting stock in the nursery, and obtaining good seedling survival and growth after planting, has been widely recognised by nursery stock managers. Mycorrhizae occur in 83% dicotyledonous and 79% monocotyledonous plants (Trappe, 1987). All gymnosperms are reported as mycorrhizal (Newman and Reddell, 1987). Although numerous fungi are able to form mycorrhizae in a particular host tree, the species composition of the mycorrhizal flora is not constant, and a temporal shift in species and numbers of fungi may occur with the age of the plants (Dighton and Mason, 1984). Based on infection morphology, mycorrhizae have been grouped into ecto- and endo-mycorrhizae.

Ecto-mycorrhizae

Ecto-mycorrhizae are the most common among forest and ornamental tree species belonging to Piniaceae, Salicaceae, Betulaceae, Fabaceae and Tiliaceae, as well as in some members of Rosaceae, Leguminaceae and Juglandaceae (Meyer, 1973). The known fungi involved in the formation of ecto-mycorrhizae are largely Basidiomycetes, but an increasing number of Ascomycetes, fungi imperfecti and Zygomycetes have been added to the list (Trappe, 1962, 1982; Miller, 1982a,b). The most important fungus is *Pisolithus tinctorius*, which can be cultured in the medium. Considerable work on the inoculum production and methods of inoculation of forest nurseries has been done in U.S.A. and a commercial preparation of *P. tinctorius*, under the trade name 'Mycorrhiz' is now available (Marx *et al.*, 1982). Besides, common fungi associated in ecto-mycorrhizae are *Amanita*, *Boletus*, *Inocybe*, *Lactarius*, *Thelephora*,

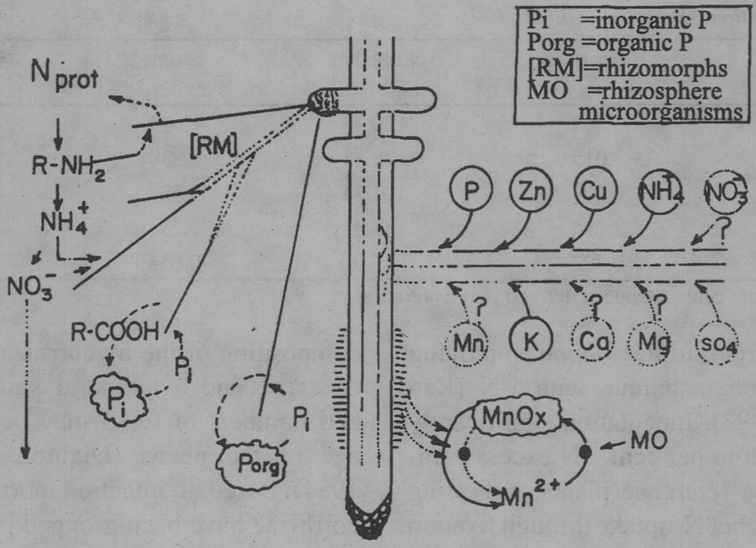

Fig. 1. Schematic diagram showing the uptake of various nutrients as influenced by VAM-fungi.

Rhizopogon, *Scleroderma*, etc., belonging to Besidiomycetes. These fungi form thick mantel or sheath around the feeder roots with mycelial strands radiating into the soil, thus enhancing the root surface area. Ectomycorrhizal roots are generally short, swollen, dichotomously branched with distinctive colours.

Besides, this fungus promotes assimilation and absorption of required nutrients, increases water absorption, reduces the absorption of toxic elements and apparently reduces the overall stress inherent on the sites (Marx and Cordell, 1988). Mikola (1973), emphasized the necessity of mycorrhizal inoculation in man made forests. Further, significance of ecto-mycorrhizae on tree growth and establishment on mined spoils was stressed by Berry (1982) and Thapar (1988). Most of the work on ectomycorrhiza is restricted to pines, with very little work on tropical trees. However, the use of this fungus is not explored for afforestation programmes in normal as well as degraded lands.

Vesicular-arbuscular mycorrhizal fungi

Vesicular-arbuscular mycorrhizae (VAM), otherwise referred to as endo-mycorrhizae, occur over a wide range of ecosystems from aquatic to desert environments (Mosse *et al.*, 1981, Kiran Bala *et al.*, 1989). However, VAM fungi are predominant amongst all species of plants (Harley and Harley, 1987). VA-mycorrizae are formed by non-septate phycomycetous fungi belonging to the genera *Glomus*, *Gigaspora*, *Acaulospora* and *Sclerocytis* of the family Endogonaceae of Zygomycetes (Gerdemann and Trappe, 1974; Trappe, 1987; Hall, 1984). These fungi are obligate symbionts and have not been cultured on nutrient

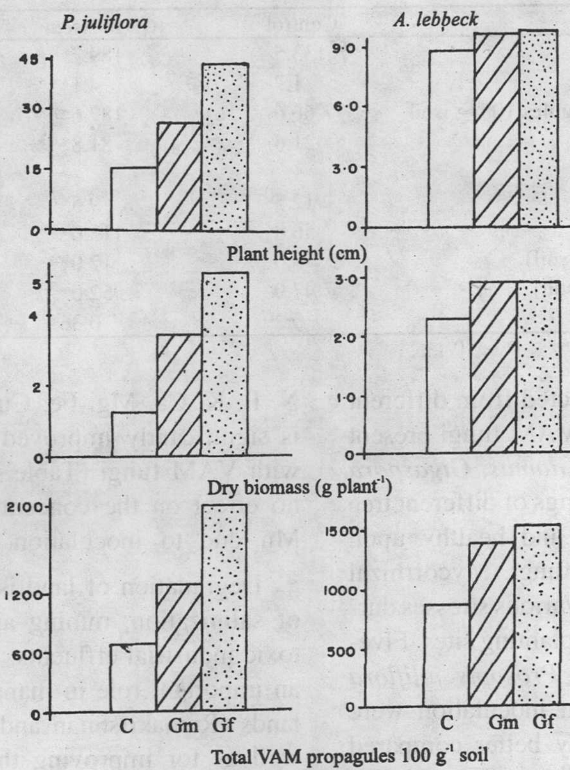

Fig. 2. Growth and dry matter production of trees as influenced by VAM-fungi (C: control; Gm: Glomus mosseae; Gf: G. fasciculatum).

media unlike ecto-mycorrhizal fungi. VAM infection has been shown to increase water and nutrient uptake and drought tolerance of several plant species (Safir and Nelsen, 1985; Simpson and Daft, 1990; Rao and Tarafdar, 1993; Tarafdar and Marschner, 1994) besides increased nutrient uptake by mycorrhizal plants (Fig. 1). The other beneficial effects are ability to reduce nitrate to ammonia for further assimilation (Ho and Trappe, 1975), transport of N from soil to the host plant (Ames *et al*., 1983), protection of host roots from diseases (Cooper, 1984) and in overcoming detrimental effects of high concentration of salts. In view of this, VAM fungi play a significant role in enhancing the establishment and productivity of tree species in arid and semi-arid regions, where plant productivity is limited due to low soil fertility and frequent droughts.

VAM fungi were found to be associated with different species of trees growing in Indian desert (Kiran Bala *et al*., 1989). Their infections are common even in xerophytes such as *Opuntia* spp. and *Euphorbia* spp. (30-100%) rate of infection. The intensity of VAM fungal infection varied among different plant species collected from the same locality, as well as within the

Table 3. Biochemical parameters in the rhizosphere of P. juliflora (2-year-old) grown in field

Parameter	Control	*G. mosseae*	*C. fasciculatum*
Plant height (cm)	133.5	189.7***	207.0***
Dry matter (kg plant^{-1})	1.7	4.1***	5.0***
No. of viable VAM propagules/100 g soil	66.6	187.6***	299.5***
Root infection (%)	21.6	81.8***	84.9***
Microbial population			
Fungi (x 10^3 g^{-1} soil)	12.5	9.5*	14.5
Bacteria (x 10^4 g^{-1} soil)	56.8	113.0***	136.5***
Actinomycetes (x 10^4 g^{-1} soil)	8.0	12.0**	21.0***
Nitrosomonae (x 10^2 g^{-1} soil)	47.0	52.0*	50.0
Organic matter (%)	0.29	0.36*	0.38**

* $P < 5\%$, ** $P < 1\%$, *** $P < 0.1\%$.

same plant species collected from different locations. Most of the VAM fungi present in arid soils belong to *Glomus*, *Gigaspora* and *Acaulospora*. Seedlings of different tree species become sturdy and healthy upon inoculation with VAM-fungi. Mycorrhizal seedlings can withstand various stresses during transportation to the planting sites. Five-month-old seedlings of *Prosopis juliflora* and *Albizia lebbek* upon inoculation were found to be significantly better compared to non-mycorrhizal seedlings (Fig. 2). Uptake of both macro and micro nutrients, except sodium and manganese by these plants, was significantly improved upon VAM inoculation. The microbial status of the rhizosphere soil of 2-year-old *P. juliflora* under field conditions, is significantly improved compared to that of the rhizosphere of non-mycorrhizal plants (Table 3). The concentration of different nutrients such as N, P, K, Ca, Mg, Fe, Cu and Zn by trees is significantly improved when inoculated with VAM fungi (Table 4). But there was no effect on the concentration of Na and Mn due to inoculation.

Degradation of land is the consequence of salinization, mining and the release of toxic industrial effluents. VAM fungi plays an important role in management of forest lands (Ramakrishnan and Pandy, 1988) as well as for improving the rangeland productivity in drastically disturbed ecosystem or restoration of vegetation in disturbed rangelands (Wali, 1988). It has been demonstrated that the presence of VAM fungi is critical for the regeneration of natural ecosystems in arid lands. Inoculation techniques for rehabilitation have been tested and are considered highly valuable, but until improved techniques of inocula production are developed, the best alternate

Table 4. Concentration of different nutrients in foliage of Prosopis juliflora (2-year-old) inoculated with VA mycorrhizal fungi

	Major nutrient concentration (mg g^{-1})						Micronutrient concentration (µg g^{-1})			
	N	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
Control	27.2	0.6	19.5	77.9	10.4	4.9	1098	24.0	11.0	24.4
G. mosseae	30.7	0.9	24.5	84.6	13.3	4.2	1191	29.0	10.9	33.7
G. fasciculatum	31.9	0.9	25.6	87.2	14.3	6.0	1295	31.5	9.9	33.6
LSD (P=0.01)	0.2	0.1	1.9	2.3	1.0	NS	85.2	2.1	NS	3.1

Table 5. Growth of P. juliflora (16 week) with dual inoculation in a sandy loam amended with NaCl (Dixon et al., 1993)

Symbiont	Total wt. (g plant^{-1})	Root wt. (g plant^{-1})	Leaf area (cm^2)	Conc. of P (%)
At 0 mM NaCl				
Rhizobium	8.8	2.3	676	0.11
VAM	12.6	3.1	960	0.16
Rhizobium+VAM	13.0	3.4	931	0.18
At 40 mM NaCl				
Rhizobium	7.6	2.1	631	0.10
VAM	10.4	3.0	923	0.16
Rhizobium+VAM	12.7	2.8	612	0.17
At 80 mM NaCl				
Rhizobium	7.1	2.2	472	0.10
VAM	8.5	2.2	639	0.16
Rhizobium+VAM	10.4	2.1	611	0.18

is a proper management of a site, preventing loss and maintenance of appropriate plant cover, where the plant roots serve as an energy source and medium for VAM proliferation. The useful employment of mycorrhizal symbiosis in this dawning era of bio-technology requires accelerated investigation on all aspects of mycotrophy, and a search for ways to avoid reduction of mycorrhizal populations, to breed crops for mycorrhizal responsiveness, and to find methods for the introduction of effective mycorrhizal fungi.

Dual inoculation by Rhizobium and VAM-fungi

Legumes require adequate phosphorus supply for satisfactory nodule production and nitrogen fixation (Van Schreven, 1958). VAM fungi are known to improve phosphorus nutrition of plants, particularly in soils containing low phosphorus (Tinker, 1980). Dual inoculation of legumes with VAM fungi and *Rhizobium* exerts synergistic beneficial effect on growth of legumes. It is attributed to the improvement in nutrient status of the host, especially P, Zn and Cu by VAM and nitrogen fixation by *Rhizobium* (Crush, 1974; Subba Rao *et al*., 1986). Establishment and growth of different NFTs can be enhanced with dual inoculation of VAM fungi and *Rhizobium* (Dela *et al*., 1988) besides significant improvement in the uptake of nitrogen and phosphorus. Umali-Garcia *et al*. (1988) observed that dual inoculation with VAM fungi and *Rhizobium* improved nodulation, nitrogen fixation and mycorrhizal colonization resulting in higher biomass production of *Leucaena*, *Acacia* and *Albizia* in a P-deficient soil compared to single inoculation with either of the organisms. Further, Dixon *et al*. (1993) reported that dual inoculation with VAM fungi and *Rhizobium* helps in mitigating the adverse effects of sodium chloride on *Leucaena* and *Prosopis* (Table 5). Phosphorus concentration in *P. juliflora* was significantly improved compared to either of the inoculations.

Transfer of N from legume to a non-legume

It is widely accepted practice to grow non-leguminous plants (grasses/millets) in between legumes as in silvopastoral

Table 6. Amount of nitrogen transferred from Leucaena to Cenchrus grass (Rao and Giller, 1993)

Treatment	Per cent legume N recovered in grass	Amount of N transferred (mg)	Per cent grass N obtained from legume
No-detopping	3.07	2.75	28.02
Detopping plus incorporation	3.51	2.37	29.88
Detopping (2 weeks)	3.87	2.11	31.63
Detopping (4 weeks)	3.59	2.11	28.40

and agroforestry systems. Legume component in these systems helps in improving soil productivity. Legume trees provide nitrogen to the grasses growing alongside, either directly through the fixation, transfer of fixed N or indirectly by non-competing for soil N. Ta and Faris (1988) reported better growth of timothy when grown along with alfalfa, compared with a mono-culture. Nitrogen accumulation in grasses was improved when grown together with *Leucaena* compared with the mono-cultured plants, besides significantly higher biomass production. Through foliar feeding techniques with ^{15}N, it was found that about 3.07 to 3.87% of legume N is transferred to grass when grown together (Table 6), while Burity *et al.* (1989) observed a transfer of 5% N fixed in alfalfa to the associated grass. Brophy *et al.* (1987) and Rao and Giller (1993) observed that about 25 to 30% of grass N is derived from the legume. In this connection, it is worth mentioning that VAM-fungal strains further help in translocating nitrogen from legume to non-legume (Haystead *et al.*, 1988).

Conclusions

Interest in applied areas of research on microbial associations has increased significantly during past few years. There is sufficient evidence of improving the tree seedlings establishment and growth upon inoculation with efficient strains of *Rhizobium* or with any other beneficial micro-organisms such as mycorrhizal fungi. Though there are opportunities to apply the microbial technology to seedling production and afforestation programmes, both in degraded lands and reclamation sites, research efforts need to be directed towards finding the appropriate tree-microbe combination adopted to well defined soil types and climatic conditions, the cultural techniques and suitable protocols in afforestation programmes on different sites. Trees and micro-organisms are evolved together by adopting to natural and man-made stresses. So, further programme aimed at selecting suitable organisms to improve stress management systems are needed. Regardless of the selected alternatives, nursery men, field foresters and planters must be aware that they are dealing with two living organisms in a symbiotic relation. Both must be nurtured to provide seedlings of superior quality for field plantations. A major benefit of microbial associations is to help the trees in better establishment and improve the productivity of the arid and semi-arid regions.

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