Vesicular-arbuscular mycorrhizae and their significance in forestry

MEHROTRA M. D.*

The term mycorrhiza (fungus-root) was Coined by A.B. Frank, a German Forest Pathologist in 1885. It functionally represents mutualistic or symbiotic association between the plant roots and nonpathogenic soil fungi by which both the partners are benefitted due to bidirectional flow of nutrients.

Mycorrhizae are broadly classified in to (i) ectomycorrhizae (2) endomycorrhizae and (3) ectendomycorrhizae of which the last one is not of much relevance to forestry. Ectomycorrhizae have restricted distribution in the plant kingdom. All the members of Pinaceae and majority of the plants of Betulaceae, Fagaceae and Dipterocarpaceae, are ectomycorrhizal. The plant families, Caesalpiniaceae, Juglandaceae, Myrtaceae and Salicaceae include both ecto and endotrophic genera. Some important tree genera, i.e. *Alnus*, *Eucalyptus*, *populus* and *Salix* are both ecto and endomycorrhizal. The ectomycorrhizae are characterized by the change in morphology of the feeder roots, presence of fungal sheath (mantle) around them and Hartig net in the outer cortex formed by the intercellular hyphae. About 5000 fungi belonging to Basidiomycetes and Ascomycetes are known to form ectomycrrhizae in about 2000 woody plants.

Endomycorrhizae include ericaceous mycorrhizae, orchidaceous mycorrhizae and vesicular-arbuscular mycorrhizae (VAM) of which VAM are of relevance to forestry. Vesicular-orbuscular mycorrhizae have a wide host range unlike ectomycorrhizae. About 90% of 30000 vascular plants including agricultural crops, fruit and forest trees develop this type of mycorrhizae. They also have a broad ecological range. They are distributed in plants from arctic to tropical regions in most ecosystems such as dense rain forests, open woodlands, heaths, sand dunes and semi-deserts. They are characterized by practically no change in the morphology of roots and the presence of special structures such as vesicles and arbuscules in the outer cortex formed by the intracellular hyphae. The vesicles cotain stored food material chiefly carbohydrates and are regarded as temporary storage organs and also serve as reproductive structures. Arbuscules are finally branched hyphal structures and they act in reverse manner to haustoria by releasing the nutrients (chiefly poly phosphates) to host cells in exchange for carbon. They are short-lived The fungi that form VA mycorrhizae belong to the family Endogonaceae of the class Zygomycetes. So far six genera of this family are known to form mycorrhizae. They are *Acaulospora*, *Entrophospora*, *Cigaspora Clomus*, *sclerocystis and Scutellospora*.

Isolation and Quantification of VAM Fungi/ propacules

The VAM fungi (spores) are isolated from the soil by collecting soil close to a plant from a depth of 10-15 cm after removing or scraping away the top cm or two. The plant is not to be pulled out as the root cortex is likely to be stripped off taking VAM infection with it. For representative sampling of the site, bulk samples of up to15 sub-samples are replicated three times. The VAM spores are isolated from the soil samples by wet sleving and decantling technique(Gerdemann and Nicolson, 1963), for which sieves of different pore sizes are used, and the spores retained on the sieves (from 50-250 pore size) examined. Majority of the spores are retained on the sieve of the pore size 100 cm. The other methods for spore isolation are Flotation and Bubbling Technique (Furlan and Fortin, 1975), Density Gradient Centrifugation Techniquc (Ohms, 1957. Mertz 1979) and Sucrose Centrifugation Technique (Jenkins, 1964) which ensure isolation of

*Division of Forest Pathology
Forest Research Institute
P.O. New Forest
Dehradun-248006 (U.P.)

spores without much foreign materials. Very recently Ciovanni-Paciconi (1992) has modified the wet sieving and decanting method to remove clay particles and debris from the spores by making use of sodium pyrophosphate (0.IM) and anti-foam agent (Tween 80, 0.1-0.5%) respectively. He has also recommended use of nylon filters instead of metallic sieves for better recuperation of VAM spores and their examination.
Flotation

Quantification of VAM propagules from soil is done by pipetting out the extracted spores in water into an eelworm counting slide of I ml capacity. In case the species of mycorrhizal fungi are to be compared or viability and infectivity of spores is to be determined, the most Probable Method (Porter, 1979, Powell, 1980) may be used. For staining the VAM infection, the standard method as described by Phillips and Hayman (1970) is used.

The VA mycorrhizal fungi have so far not been cultured on artificial or synthetic media. This is the man Problem with this group of fungi as in the absence of axenic culture it is not possible to know their actual physiological behaviour and the metabolites they elaborate. These fungi can grow only in association with the living roots. They produce a large number of spores in the soil on the extramatrical mycelium which is developed profusely once the fungus enters into symbiotic association with the host plant.

Production of VAM Inoculum

The VAM inoculum is used to produce quality seed lings. The seedlings are fortified with symbiotically efficient VAM fungi for their establishment and improved growth particularly in harsh or critical sites. The VA mycorrhizal fungi are not host specific but show host preference, hence the need for selection of suitable fungi to confer maximum benefits to plants. The first step to produce high quality VAM inoculum is to obtain symbiotically potential strains of the fungi from a reliable source within the country or a foreign agency. The most accessble source of inoculum for starter culture is the rhizosphere of endomycoorrhizal plants in the field. Collection for native VAM fungi are made from different areas under the same tree species and sui table ones are selected by screening. This is necessary as the VAM fungi from unrelated host genera from distant areas may not be beneficial to the plants.

The spores after isolation from the soil by wet sieving and decanting technique are strilized by immersing in a solution containing 2% (w/v) chloramine T and 200 ppm streptomycin for 15 minutes followed by washing three to four times in sterilized water.

The spores multiplied separately for each species sterilized soil or artificial substrate such as soilrite· perlite mixture by growing a cover crop preferably maize or sorghum. These crops are good for producing VAM inoculum because they show high susceptibility to VAM colonization owing to rapidly growing fibrous root system. The spores germinate and colonize the roots and in about 4-6 months the fungus establishes its hyphal- soil network and produces a good number of spores. The other plants which are used as cover crops are Sudan grass. Rhodes grass, Conchrus gras ,Coleus, Clover etc. During winter months culturing of VAM fungi may be done on carrot too as it has been found to support a good spore population. The following are the optimum conditions which are desired to be maintained at the VAM prodution unit.

Soil : sand (1:1) or soilrite : prelite (1:1) mixture which provides good drainage and aeration. The mixture is sterilized with methyl bromide as fumigant prior to inoculation with the VAM spores as it reduces competition for the introduced VAM fungi fromsoil microflora including the indigenous VAM fungi.

Soil pH between 5 and 6 : temperature 25—28° C, relative humidity between 70-80%, large-sized pots which support good growth of plants with extensive root system; constant supplemental low fluorescent or incandescent light for about 14h/day and 75-100 ppm phosphorus level in the substrate are the ideal conditi ons for bulk culturing.

Captan and Furdon 3G are recommended to control saprophytic fungi and nematodes respectively without any adverse effect on inoculum production and plant growth.

On harvesting the cover crop, the roots are cut into pieces and mixed with the soil thoroughly to have homogenous inoculum. The inoculum consisting of soil, root fragment and fungal spores may be applied in the nursery by any of the following methods: broad-

acsting, banding and side dressing, placing in layers or pads directly beneath seeds or pelleting of seeds. About 300-500 spores per pot (500g soil) and approximately 10,000 spores per m^2 of planting area are used to ensure rapid colonization of roots and good mycorrhization of seedlings.

Criteria for Selection of VAM fungi

The main criteria for selection of symbiotically efficient VAM fungus (SEVF) for a given host plant are : its ability to colonize the roots extensively, to form maximum number of arbucules (for bidirectional flow of nutrients)and extensive extramatrical mycelium in the soil or mycorrhizosphere contributing greatly to increased uptake of nutrients from the soil and enhancement of plant growth. Superior plant height, stem diameter, more branching, leaves and higher dry weight of mycorrhizal seedlings make them qualitaively superior and thus enable them to withstand odd conditions of stressed sites resulting in better establishment and higher percentage of survival in outplantings besides ensuring improved growth of the planted seedlings thereafter. This is what is required in plantation forestry and can be achieved through mycorrhization of seedlings with symbiotically efficent mycobionts.

Significance of VAM in forestry

Vesicular-arbuscular mycorrhizae confer many benefits to plants. In mycorrhizal association, a large portion of the fungal body remains outside the root in the form of extramatrical hyphae which ramify in the soil exploring for nutrients and water, This network of hyphal extension in the soil increases the absorptive surface of roots tremendously and thereby increases uptake of nutrients and plant growth. VA mycorrhizae help the plants grow in infertile soil because of the weathering ability of VAM fungi. These fungi play key roles in mineral cycling energy flow and plant succession in disturbed and undisturbed ecosystems. They make available immobile or fixed form of phosphorus as a result of solubilization by organic acids which the fungi elaborate as a part of their normal metabolic activities. Besides, they also make available phosphorus from organic phosphates in forest litter. They are also known to make available other elements such as nitrogen, sodium, magnesium, zinc, copper calcium etc. VA mycorrhizae reduce fertilizer and this is of great importance.

Vesicular-arbucular mycorrhizae increase tolerance of the plants to odd conditions such as high soil temperature, poor availability of water, drought, extreme of soil acidity and heavy metal toxicity and thus help them establish and survive better in critical or harsh sites. They decrease transplanting shock and thereby increase survival percentage of seedlings in outplantings leading to success of plantations degraded areas. They are also known to alleviate soil compaction and thus improve soil porosity and aeration. Besides, they are helpful in binding sand into semistable aggregates and this is of importance particularly in semi-desert situations. In leguminous tree species they are reported to increase the activity of nitrogen fixing organisms in the root zone.

VA mycorrhizae are also known to control root diseases and plant parasitic nematodes. Their role in biocontrol of diseases is not direct but indirect. Increased lignification of the cell wall and high chitinolytic activities are reported to check invasion of roots by the pathogens and nematodes. Poor root exudation at high phosphorus level in the roots does not favour proliferation of root pathogenic fungi and thereby helps in obviating fungal attack on roots. Also high amino acid content in the roots especially arginine is reported to influence the rhizosphere architecture through root exudation which has suppressive effect on pathogenic fungi. Increased production of plant protection phytoalexins (isoflavenoid) attributed to the symbiosis.

In tropical countries like India there is a problem of nitrogen deficiency and phosphorus non-availability and this problem is accentuated particularly in degraded or stressed areas. Fertilization of soil in forestry practice is not possible because of economic considerartions and, therefore, it is imperative to make use of biofertilizers as a substitute or inorganic fertilizers. In view of the above attributes, the VA mycorrhizal fungi confer to the plants they are good candidates for use as biofertilizers as well as biocontrol agents. They are safe to be used as they are non-obnoxious, non-polluting and non-hazardous.

The research carried out in India and abroad has shown the significant role the VAM can play in stimulating plant growth. Also their role in biological rejuvenation of wastelands has been emphasized. The

research on VA mycorrhizae at Forest Research Institute, Dehradun was started in early seventies and considerable amount of work has been done. Besides determining the endomycorrhizal status of important tree species and distribution of VAM fungi in forest soils, the stimulatory effect of VAM and *Rhizobium* as coinoculants has been demonstrated in *prosopis juliflora* and *Acacia nilotica.* Further, *Agathis robusta* and *Araucaria cunninghamii* have also been found to respond positively to VAM inoculations. Currently, efforts are being made to isolate and screen VAM fungi for their suitablity to different tree species. The inocula of different VAM fungi are being maintained. The technique for bulk culturing of VAM fungi standardised at the Institute will help a long way in producing sufficient inoculum for producing quality seedlings in the nursery suited to critical sites which are difficult to revegetate or degraded sites with poor forest productivity.

In recent years suitable technology has been developed at Forest Research Institute, Dehra Dun for macropropagation of seedlings of bamboo, eucalyptus and *Casuarina* and a large number of seedlings can now be produced vegetatively. The VAM technology developed at the Institute is suited to *Macropropagated* seedlings as well. The seedlings can be mycorrhized economically with small quantity of inoculum of suitable VAM fungi to optimize their growth. Mycorrhization of *Macropropagated* seedlings can protect them from root pathogens and also ensure better establishment and survival in outplantings. As eucalyptus and *Casuarina* are now being raised on a short rotation of 7-10 years to meet the demand of raw materials for paper industry, improvement in plant growth through mycorrhization with suitable VAM fungi will brighten the prospects of increased biomass production.

References

Furlan, V. and J.A. Fortin. 1975. Naturaliste Can., 102:663-667.

Cerdeman, J.W. and T-H. Nicolson. 1963. Trans. Brit. Mycol. Soc., 46:235-244.

Jenkins., W.R. 1964. Plant Dis. Rep., 48:692.

Mertz, S.M., J.H. Heithaus and R.L. Bush. 1979. Trans Brit. Mycol. Soc., 72:167-169.

Ohms, R.E. 1957. Phytopathology, 47:751-752.