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Short communication

Ectomycorrhizal Formation in Micropropagated Plantlets of *Populus deltoides*

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Abstract

Five ectomycorrhizal fungi, *Cenococcum geophilum, Laccaria laccata, Paxillus involutus* and two isolates of *Pisolithus tinctorius* were used to inoculate micropropagated plantlets of *Populus deltoides* (G 48) to produce the mycorrhizas *in vitro. Paxillus inovlutus* formed mycorrhizas with plantlets of *P. deltoides* while others failed, though they have colonized the substrate extensively. The plantlets colonized with *P. involutus* significantly showed more growth and dry weights when compared to other fungi.

Keywords: Poplar, micropropagation, *Paxillus involutus, in vitro* synthesis

Poplar grow satisfactorily on soil types of varying fertility (Harris and Jurgensen, 1977). Different species of poplars were introduced in India to meet the growing demand for wood and because of their rapid growth rate and

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ability to coppice, which are valuable characteristics for their utilization in short rotation forestry. Efficient mycorrhizal symbionts on poplar could improve establishment of plantations and utilization of available nutrients. Although ectomycorrhizas, endomycorrhizas and ectendomycorrhizas have been recorded on poplar species (Dominik, 1958; Vozzo and Hacskaylo, 1974) the ectomycorrhizas of poplar are not well documented. Trappe (1962) recorded 31 ectomycorrhizal fungi in association with poplars, the most commonly observed species among these being *Amanita muscaria* (L.Fr) pers. ex Hooker. *Boletus edulis* Bull.: Fr., *Hebeloma crustuliniforme* (Bull.:Fr.) Quel., *Russula delica* Fr., *Paxillus involutus* (Batsch) Fr. and *Cenococcum qraniforme* (Sow.) Fred. & Wings. Several species of poplars are propagated through tissue culture to produce more plants which are difficult to propagate rapidly by conventional methods. Micropropagated plants require a long period of transition to become adapted to *ex vitro* conditions and are affected by water stress due to the low nutrient absorption capacity of their roots (Flick et al., 1983). The presence of mycrorrhizas could increase the availability of limiting nutrients such as phosphorus and nitrogen by facilitating their absorption (Martins et al., 1996), and also enable them to acclimate to *ex vitro* conditions. It is therefore necessary to ensure that these plants form effective mycorrhizas. In the present investigation, an attempt was made to inoculate micropropagated plantlets of a single clone of *Populus deltoides* (G 48) with some ectomycorrhizal fungi in order to identify the strains that can form ectomycorrhizas.

Ectomycorrhizal fungi, *Cenococcum geophilum* Fr. (isolate from Indonesia), *Laccaria laccata* (Scop.) Berk & Br. (isolate KN12 of *Pinus patula*, India). *Paxillus involutus* (Batsch) Fr. (isolate 69 of *Populus euramericana*, France), and 2 isolates of *Pisolithus tinctorius* (Pers.) Coker and Couch (one isolate KN6 of *Eucalyptus tereticornis*, India and the other Pt144 from *Quercus* sp., USA) were used in this study. All the cultures were maintained on modified Melin Norkrans medium (MMN) (Marx, 1969). The micropropagated plantlets of *Populus deltoides* Marsh (Clone G 48, introduced from America) were obtained from the Tissue Culture Pilot Plant, Tata Energy Research Institute, New Delhi, India. The plants were 1.5–2.0 cm tall with young leaves and transferred to fresh MS medium for rooting once obtained. In 500 ml glass jars, 120 ml of vermiculite and soil rite (2:1) was filled in each jar to which 60 ml of root initiating medium [half strength MS basal medium with 1% sucrose (Murashige and Skoog, 1962)] was added and autoclaved at 121°C for 40 min. The plantlets of *P. deltoides* were transferred to the jars after growing them for a week in MS medium for rooting, i.e., at the root initiation stage. The plantlets were grown for 2 weeks at 25°C under 16h light and 8h dark. They were then inoculated with 3 mycelial discs (8 mm diameter) of test fungi cut from the edge of the actively growing mycelium. The plants were well

established in the jars when the fungi were inoculated. The plants were maintained under the growth conditions described above. Ten replicates were maintained for each host/fungus combination. Twelve weeks after inoculation, the plants were harvested and assessed for growth and mycorrhizal formation. Short roots with complete development of a fungal mantle were scored as mycorrhizas. Roots were hand sectioned to confirm the mycorrhizal structures. Roots associated with the fungi were fixed in 2.5% gluteraldehyde and microscopic characterization was done by taking transverse sections (1–1.5 mm) on a Ultracut E microtome and stained with trypan blue in lactophenol. Four to five mycorrhizal short roots were randomly selected from each replicate and the microscopic characterization was studied. Plants were measured for stem height and the dry weights were taken after drying the shoots and roots at 80°C for 48 hr. The data were subjected to analysis of variance and the means were compared by Bonferroni's multiple comparison test. The experiment was repeated under the same conditions to confirm the obtained results.

All the fungi grew well and were found to colonize the substrate extensively in the root initiating medium. Among the fungi tested, only *P. involutus* formed ectomycorrhizas and the other fungi such as *C. geophilum, L. laccata* and two isolates of *P. tinctorius* failed to initiate mycorrhizas with *P. deltoides* even though these fungi colonized the substrate extensively. The mycorrhizas were characterized by enhanced root branching, the presence of a mantle and Hartig net. *P. involutus* formed mycorrhizas in all the replicates inoculated and colonized 73% of the lateral roots. The mycorrhizas formed by *P. involutus* are simple (1.9–2.0 × 0.4–0.5 mm) with numerous irregularly spaced branches, typically occurring along one main axis. The mycorrhizas are pale white to yellowish when young and turned brown on aging. The mantle thickness ranged from 20–40 μm with an outer layer of prosenchymatous hyphae and an inner layer of synenchymatous hyphae. The Hartig net showed uniform intracellular penetration by hyphae up to two cortical cell layers deep. The plantlets inoculated with *P. involutus* showed significant improvement in height and dry weights of shoot and root when compared to those with other fungi (Table 1). Only *P. involutus* formed mycorrhizas when the experiment was repeated under the same conditions and the remaining fungi failed to form mycorrhizas with *P. deltoides*.

Many *in vitro* synthesis mycorrhizal systems have been developed to synthesize mycorrhizas with different tree species. In many of these systems, either MMN or modified fungal growth media was used to synthesize ectomycorrhizas. In the present study root initiating medium was used instead of MMN medium to develop mycorrhizas and the root system simultaneously in micropropagated plantlets of *P. deltoides*. Malajczuk and Hartney (1986) reported rapid fungal growth and mycorrhizal formation in micropropagated plantlets of *Eucalyptus camaldulensis* Dehnh. with different fungal isolates of

Table 1. Effect of different ectomycorrhizal fungi on the growth and mycorrhizal development of micropropagated plantlets *Populus deltoides*

Fungus	Shoot height (cm)	Shoot dry wt. (mg)	Root dry wt. (mg)	Shoot/root ratio	No. of mycorrhizal short roots
Cenococcum geophilum	4.2b ±0.2	28.7b ±4.25	38.5b ±3.3	0.77ab ±0.12	ND
Laccaria laccata	4.4b ±0.22	44.8b ±5.78	99.2b ±27.3	0.74ab ±0.15	ND
Paxillus involutus	7.4a ±0.5	75.6a ±10.7	194.8a ±38.7	0.63ab ±0.16	122.0 ±18.2
Pisolithus tinctorius (KN6)	5.0b ±0.4	30.0b ±4.3	35.0b ±3.8	0.89a ±0.12	ND
P. tinctorius (Pt144)	4.8b ±0.34	37.3b ±2.9	131.0b ±17.8	0.32b ±0.16	ND

The columns sharing the same letter are not significant at $P<0.05$. The values are mean of 10 replicates followed by standard error of mean. ND = not detected.

P. tinctorius, *Scleroderma* spp., *Hysterangium* spp., and *L. laccata* in the root initiation medium. From their results it was concluded that the inoculation of micropropagated plantlets with selected ectomycorrhizal fungal isolates is possible on either root culture medium or fungal growth medium. They have also suggested that inoculation of ectomycorrhizal fungi at the root initiation helps the fungus to precolonize on the root surface thus avoiding competition and/or antagonism from the general soil microflora when they were transplanted to outplantation sites. The use of genetically identical clones in studies of mycorrhizal formations in poplar is desirable since clones of selected hybrids are extensively used in commercial plantation. *Populus* hybrids show extensive selectivity in mycorrhizal formation (Heslin and Douglas, 1986). The formation of mycorrhizas of *P. deltoides* with *P. involutus* support the view that this fungus has a broad host range at the species level and at the isolate level. The broad range of hosts for *P. involutus* was previously reported by Molina and Trappe (1982). In addition, this species has been reported to form mycorrhizas *in vitro* with seedlings of *Populus tremula* (Laiho, 1970), *P. euramericana* (Fontana, 1961) and *Populus hybrid* TT32 (*P. trichocarpa* and *P. tacamahaca*) (Heslin and Douglas, 1986). The isolate of *P. involutus* used in the present study colonized extensively the feeder roots of *P. deltoides*

plantlets and formed a thick mantle and uniform Hartig net. Heslin and Douglas (1986) reported variation among the isolates of *P. involutus* in forming mycorrhizas with *Populus* hybrid TT32. They tested four isolates of *P. involutus* (from poplar and *Picea sitchensis*) with *Populus* hybrid TT32 and found that one isolate (from poplar) showed a fragmentary mantle and non-uniform Hartig net, while other isolates form an uniform Hartig net and thick mantle. The plantlets colonized with *P. involutus* showed more root dry weight than those with other fungi, supporting the results of Navratil and Rochan (1981) that mycorrhizal plants can increase the number and dry weight of roots of poplar more than non-mycorrhizal plants. Although the strain of *C. geophilum* used in the investigation was not isolated from poplar, it was selected because of its association with a wide range of poplar species (Fontana, 1961; Trappe, 1962). Heslin and Douglas (1986) had also reported that this fungus failed to form mycorrhizas with *Populus* hybrid TT32 even though one of the isolates was originally isolated from poplar. Two isolates of *P. tinctorius* were selected because of their ability to form mycorrhizas with different tree species and also its wide geographic distribution (Marx, 1977). Heslin and Douglas (1986) have observed a mantle without Hartig net with *P. tinctorius* when inoculated to *Populus* hybrid TT32. *L. laccata* also did not initiate mycorrhizas in this study despite the fact that it is known to form mycorrhizas with different tree species. Failure of this fungus to form mycorrhizas with *P. deltoides* may be due to highly developed strain specificity that is known for ectomycorrhizal fungi.

It can be concluded from the results that among the different ectomycorrhizal fungi tested, only *P. involutus* formed mycorrhizas while others failed under the described experimental conditions. Transplantation of mycorrhizal plantlets to nursery and monitoring their performance in the field can only reveal the importance of this fungus in plantation programs involving poplars.

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