NOTE

MYCORRHIZAL INOCULATION OF *HOPEA ODORATA* (DIPTEROCARPACEAE) IN THE NURSERY

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Previous experiments under laboratory conditions on ectomycorrhizal inoculation of *Hopea odorata* and *H. helferi* have shown that heavy infection could be obtained with corresponding increase in shoot dry weight by up to 7.3 times and increased foliar phosphorus concentration by about 2 times (Yazid *et al.* 1994). The experiments were conducted using cardboard inoculum (Chilvers *et al.* 1986) which is a convenient technique for screening potential mycorrhizal fungal strains. However, a prerequisite for the production of high quality mycorrhizal inoculated dipterocarp seedlings is the use of other forms of inoculum which can be mass produced cheaply and easily. A form of inoculum which is widely used in the temperate regions for inoculation of seedlings of forest tree species is a peat-moss: vermiculite mixture mixed with a suitable nutrient medium and colonised by the hyphae of selected ectomycorrhizal fungi. Thus this study was carried out to investigate the potential of a similar type of inoculum for inoculation of dipterocarp seedlings.

Two hundred millilitres of modified Melin-Norkrans medium (Marx & Bryan 1975) was added to a 300 ml mixture of chopped coconut husk fibre and vermiculite (1:9) (vermiculite inoculum) in glass jars. Coconut husk was sucessfully substituted for peat moss which is more readily available in temperate region nurseries. This substrate was then autoclaved for 20 min at 120 °C. On cooling, the substrate was inoculated with 10 plugs (5 mm diameter) of the test fungus which had been raised on agar medium (potato dextrose agar) for two weeks. *Pisolithus tinctorius* strain Pt441 obtained from a carpophore collected in Brazil under *Eucalyptus citriodora* by M.H. Ivory was used as the test fungus. The inoculated jars were kept at ambient room temperature (about 28 °C) for between 6 and 8 weeks until complete colonisation of the substrate had occurred.

Four-week-old seedlings of *Hopea odorata* Roxb. germinated in sand were transplanted into polybags containing approximately 500 g of a steam sterilised forest soil:sand mixture (3:1) (Yazid *et al.* 1994). Before transplanting, the potting substrate was inoculated by adding 20% v/v of the actively growing mycorrhizal inoculum. Two control treatments were used: C1 which consisted of uninoculated potting susbtrate and C2 with the autoclaved fungal inoculum mixed in the same proportion as in the inoculated treatment. The seedlings, 15 replicates per treatment, were kept for 6 months in a shade house with 37% relative light intensity.

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At harvest, roots of all plants were examined under the stereomicroscope for mycorrhizal infection. Control plants infected by indigenous nursery mycorrhizal fungi and inoculated plants poorly colonised by *Pisolithus tinctorius* or contaminated by other mycorrhizal fungi were discarded, leaving 10 replicates for analysis in each treatment. Shoot heights, and shoot and root dry weights were measured and compared using the Bonferroni test in Statgraphics Version 4.0 (STSC Inc. 1989).

Control plants treated with the autoclaved inoculum (C2) were 153% or 2.5 times taller than uninoculated control plants (C1) ($p = 0.05$), most probably due to nutrients supplied by the autoclaved inoculum [Figure 1(i)]. In addition, the biological activity of *P. tinctorius* further increased the seedlings' growth. Infected plants were 62% taller than plants treated with the autoclaved inoculum (C2) ($p=0.05$). The same trend was observed in mean shoot dry weight except that there was no significant difference between the two control treatments, C1 and C2 [Figure 1(ii)]. Mean shoot dry weight of the inoculated plants was significantly higher than that of the C1 and C2 plants. Mean root dry weight of the inoculated plants was significantly higher than that of C1 plants but not significantly different from that of C2 plants [Figure 1(ii)].

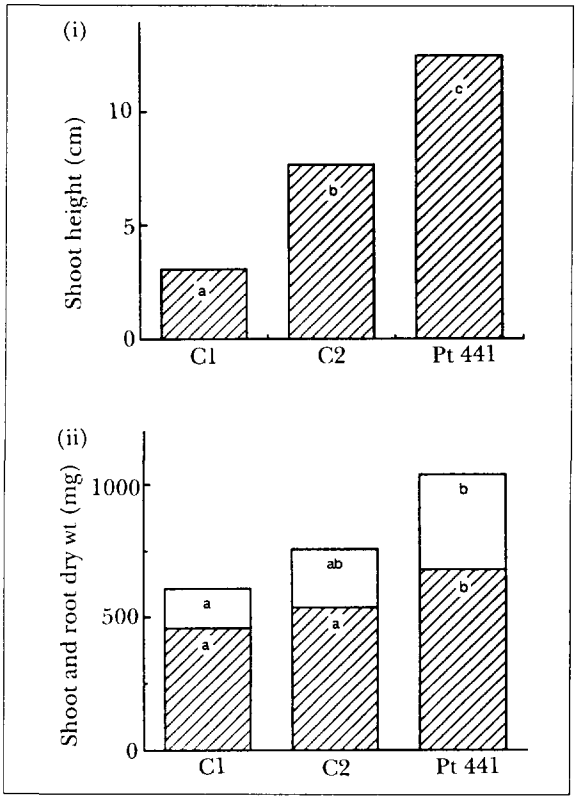

Figure 1. Growth preformance of *Hopea odorata* seedlings inoculated with *Pisolithus tinctorius* strain Pt441 grown on vermiculite compared with uninoculated control plants (C1) or plants treated with autoclaved Pt441 inoculum (C2) after 6 months: (i) mean shoot height, (ii) shoot (shaded bars) and root (unshaded bars) dry weights. Means with the same letters are not significantly different at $p = 0.05$ (Bonferronni test).

The difference in growth between the control treatments C1 and C2 strongly suggests that the fertility and/or the structure of the forest soil:sand mixture used as nursery substrate was not optimun for raising these dipterocarp seedlings. Due to the incorporation of the autoclaved inoculum (20% v/v) into the nursery substrate, the resulting substrate was lighter, and 3.5 mg of nitrogen, 11.5 mg of phosphorus and 9.3 mg of potassium were added to each seedling. The introduction of an efficient mycorrhizal fungus in addition to these additional nutrients was able to significantly stimulate seedling growth, possibly due to better substrate exploration, higher nutrient mobilisation potential and/or host plant metabolism regulation. However, extra work is still needed towards the production of high quality dipterocarp seedlings. While efficient mycorrhizal strains will equip the roots with symbionts able to extract nutrients from poor forest soils after outplanting, optimum ferlitilisation in the nursery should stimulate seedling growth without eliminating the fungus.

In Malaysia we have recently observed that the strain of *Pisolithus tinctorius* used, i.e. Pt441, may not be persistent after outplanting in logged-over forest, probably because it is not well adapted to tropical rain forest conditions (Chang *et al.* 1994a,1994b). However, the inoculation protocol proposed here should still be suitable for production of dipterocarp seedlings infected with indigenous ectomycorrhizal strains selected for their growth stimulating abilities.

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