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PURE CULTURE SYNTHESIS OF PINUS PATULA ECTOMYCORRHIZAE WITH SCLERODERMA CITRINUM

K. KANNAN and K. NATARAJAN*
Department of Botany, A. V. V. M. Sri Pushpam College, Poonodi 613 503, India.
*CAS in Botany, University of Madras, Madras 600 025, India.

Mycorrhizae of fungus-root associations are the norm for most vascular plants. The relationship between the fungus and the host is a symbiotic one, the fungus helping the host by increased water and nutrient uptake, affording tolerance to drought, soil toxicity, extreme pH and protection against root pathogens and in return the fungus obtains from the host simple sugars and vitamins.

Mycorrhizae are generally divided into two main groups the ectomycorrhizae and the endomycorrhizae. The forest trees like pines form ectomycorrhizal association with their fungal symbionts and the fungi forming ectomycorrhizae are primarily Agaricales and Gasteromycetes. Among the mycorrhiza forming Gasteromycetes, much work has been carried out mainly with Pisolithus tinctorius in USA and with Rhizopogon spp. in Australia and South Africa and only meagre information is available on the formation of pine mycorrhiza with Scleroderma aurantium, a synonym of Scleroderma citrinum Pers.

S. citrinum has been shown to be in mycorrhizal association with many species of Pinus, but Pinus patula is not one among them. The occurrence of S. citrinum in India has been reported earlier. The occurrence of fruitbodies of this species in large numbers in P. patula plantations of Kodaikanal, indicates that it is probably a mycorrhizal former with this tree. As Melin and Modess have considered that presumed hyphal connections between the sporophore and the host plant could not be taken as a proof for mycorrhizal association and only synthesis experiments under controlled conditions can furnish conclusive proof for the mycorrhiza-forming ability of a given fungus, pure culture synthesis of mycorrhiza of P. patula was attempted with S. citrinum.

The mature fruitbodies of S. citrinum were collected and the mycelial cultures were isolated from the surface-sterilized aseptically germinated basidiospores on Hagem’s agar medium and subsequently subcultured on the same medium in petri plates. Mycelial plugs were cut from the growing margin of a 21-day-old colony of the fungus and were aseptically transferred to sterilized 250 ml Erlenmeyer flasks, each containing glass beads and 100 ml of Melin-Norkrans’ solution and were placed in thermostatically controlled (temp. at 25 ± 1°C) rotary shaker to obtain mycelial suspension.

Seeds of P. patula were aseptically germinated and grown following the procedure of Ekwebelam. Seeds were surface-sterilized in 30% H₂O₂ for 1 hr, repeatedly washed in sterile distilled water, aseptically transferred to petri plates containing Hagem’s agar and incubated at 25 ± 1°C in dark. Seedlings (2 cm long) were aseptically transferred to previously sterilized 500 ml Erlenmeyer flasks, each containing 30 g of vermiculite moistened with 120 ml of nutrient solution. Each flask was inoculated with a single seeding. The seedlings were grown in a growth chamber at 20 ± 1°C with 16 hr photoperiod with light intensity at 1000 lux using cool-day tubelights.

After two months growth, the seedlings were inoculated with mycelial suspension of S. citrinum, 10 ml per flask. At the same time, 20 ml of Melin-Norkrans’ solution were added aseptically into each flask. Two months after inoculation, the seedlings were removed from the flasks, their roots washed with water and the cleaned roots were examined for the presence of mycorrhiza by using a binocular zoom stereoscopic microscope. Mycorrhizal roots were fixed in formalin-propionic-alcohol, embedded in paraffin, microtomed and 10 μm thick sections were double stained with safranin-
haematoxylin following the method of Johansen\textsuperscript{10} and examined.

In vitro growth of *P. patula* seedlings (figure 1) inoculated with mycelial suspension of *S. citrinum* resulted in the formation of short dichotomously branched lateral roots clothed with a mantle of white hyphae showing autofluorescence (figure 2). Similar white dichotomously branched ectomycorrhizae were obtained in the pure culture synthesis between *P. resinosa* and *S. auranium*\textsuperscript{3}. Individual mycorrhizae measured 1–2 mm long x 0.5 to 0.75 mm in diameter.

The anatomical structure of the mycorrhizae (figures 3 and 4) revealed the formation of a thick fungal hyphal sheath of 35–50 μm thickness over the epidermis. The Hartig-net consisted of intercellular hyphae in the epidermis could be well seen (figure 3). The occurrence of external fungal mantle and the internal Hartig-net demonstrated the formation of ectomycorrhiza in the pure culture synthesis of *P. patula* with *S. citrinum* attempted here. Various species of *Scleroderma* have been shown to form ectomycorrhizae with other species of *Pinus*\textsuperscript{11}. To the best of our knowledge this is the first reported

**Figures 1–4.** Pure culture synthesis of ectomycorrhizae of *Pinus patula* with *Scleroderma citrinum*. 1. 4-month-old inoculated seedling grown in conical flask in vermiculite-nutrient solution; 2. Dichotomously branched mycorrhizae showing autofluorescence of the fungus mantle (× 40); 3. Longisection of the mycorrhizal lateral root showing fungus mantle covering the root and Hartig-net (H) in the intercellular spaces between epidermis and cortex (× 150); 4. Enlarged view showing fungus mantle (FM) (× 300).
pure culture synthesis of ectomycorrhizae between *S. citrinum* and *P. patula* and serves as a basis for future experimentation.

Based on its abundant occurrence even during dry seasons (author's observation, unpublished) *S. citrinum* appears to have potential value in reforestation programmes in India similar to *Pisolithus tinctorius* in USA².

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**INDUCTION OF PROLINE ACCUMULATION BY METHYLPARATHION IN SORGHUM (SORGHUM BICOLOR L.)**

A. A. DESHPANDE and
G. SIVAKUMAR SWAMY

*Department of Botany, Karnataka University, Dharwad 580 003, India.*

Plants have been known to accumulate high levels of proline when subjected to water stress¹, salinity²,³, high and low temperatures⁴,⁵, nutrient deficiencies⁶,⁷, water-logging⁸, fungal infection⁹ and air pollutants¹⁰,¹¹,¹². The biochemical mechanism leading to the induction of proline accumulation and its physiological significance have not been eluci-

dated although it has been suggested that proline may be involved in osmoregulation¹³,¹⁴. Since many of the stress factors induce proline accumulation, and no information is available on the effects of pesticides with regard to proline accumulation, it was of interest to study the effect of pesticide stress in plants with reference to this physiological response. Methylparathion, an organophosphorus pesticide, is widely used as an insecticide as a spray and the residual effect of this pesticide has been known to remain in the environment for a long time¹⁷,¹⁸. Therefore, it was decided to study the action of this pesticide using both as a spray and for seed treatment since the seeds when sown are exposed to the pesticide residue in the soil.

Certified seed of sorghum (*Sorghum bicolor* L.), var. CSH-1, was purchased from the Karnataka Seeds Corporation. Technical grade methylparathion was a gift from Bayer (India) Ltd. The seeds were soaked for 1 hr in different concentrations of methylparathion (as given in the legend to figure 1), surface-washed and then sown on moist filter papers in petri dishes. Fresh and dry weights were measured after 72 hr of growth of the seedlings and the results are plotted according to Prat et al¹⁹. Proline contents were estimated²⁰ in the seedlings grown in plastic pots containing soil, at different time intervals after 5 days of initial growth and the results are plotted in figure 2. Proline estimations were terminated on the third or fourth day after the initial 5 days

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![Figure 1](image.png)

**Figure 1.** Kinetics of growth inhibition of sorghum seedlings exposed to methylparathion. [●] Untreated; ○ 100 ppm; □ 200 ppm and ▲ 400 ppm methylparathion treated seedlings. The horizontal and vertical bars represent the standard error of the mean values of fresh and dry weights respectively.]