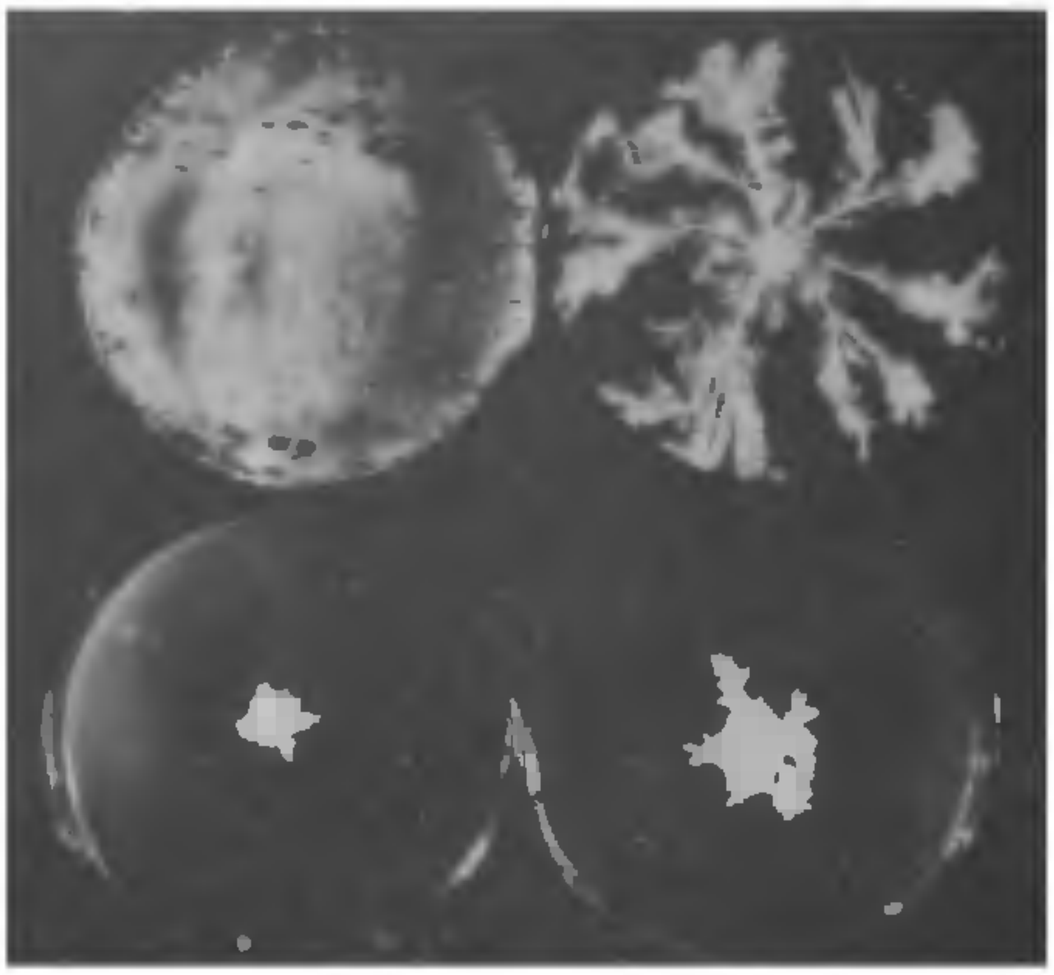

Figure 1. Effect of different concentrations (0, 1000, 5000 and 10,000 ppm) of 2, 4-dinitrophenol on *Sclerotium rolfsii* Sacc.

Colony surface became glossy and the hyphae aggregated into thin-stranded structures (figure 1). A few sclerotia of very irregular shape and size was also seen.

With 1000 ppm riboflavin, tryptophan and proline and 10,000 ppm picric acid and 2, 4–dinitrophenol, hyphal growth was almost arrested and the fungus failed to develop any sclerotia.

Picric acid and 2, 4–dinitrophenol required more than five times the concentration of amino acids and vitamins for the same morphogenetic effect. Other amino acids and vitamins did not change the morphology of the pathogen and the growth was almost similar to the control in these treatments.

The hyphae which were narrow, thin-walled and large-celled in the untreated control became short-celled and thick-walled at all the concentrations tried.

The hyphal and sclerotial characters change when a fungus is subjected to poison stress[1]. One of the remarkable features of such changes is the reduction/elimination of the ability to produce sclerotia[3].

The present results show that the test fungus can tolerate higher concentrations of 2, 4-dinitrophenol, picric acid and other chemicals. Though they are effective against a large number of microorganisms, they are effective only at very high concentration in the case of *S. rolfsii* Sacc (figure 1). Development of tolerance to a chemical by the pathogen may be due to the decrease in permeability of the fungal cell to the chemical and the conversion of the chemical into an inactive form by the pathogen[4]. It may also be due to the selective effect of the chemical which is more active only with selective fungi[1].

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IN VITRO SYNTHESIS OF ECTENDOMYCORRHIZAE OF *PINUS PATULA* WITH *AMANITA MUSCARIA*

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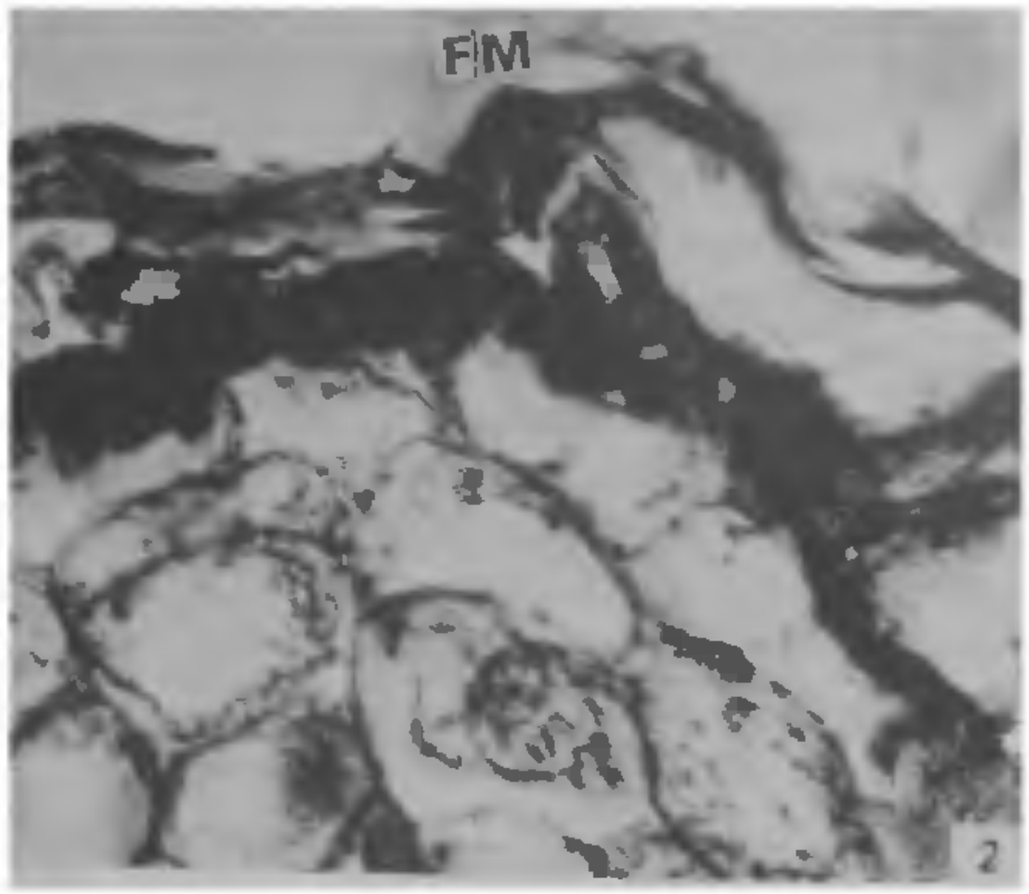
Mycorrhizae or fungus-root associations are the norm for most vascular plants[1]. Many plants depend on their mycorrhizal structures for adequate uptake of nutrients and survival in natural ecosystems[2–4]. The forest trees like pines possess ectomycorrhizal roots and the fungi producing ectomycorrhizae are primarily Agaricales and Gasteromycetes[5]. *Amanita muscaria*, a member of Agaricales is reported to form ectomycorrhizal association with many species in *Pinus*[6].

It is believed[7,8] that 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, *in vitro* synthesis of mycorrhiza of *Pinus patula* with *Amanita muscaria* was attempted.

The fruitbodies of *A. muscaria* (L. ex Fr.) Pers. ex Hooker found in association with the roots of *P. patula* Schlecht & Cham present in the New Pine plantations of Kodaikanal, Tamil Nadu were collected and used for inoculation studies. Pure mycelial cultures were isolated from the surface-sterilized stipe tissue grown on 2% Hagem's nutrient agar as modified by Modess[8] and subsequently subcultured



Figures 1 and 2. *In vitro* synthesis of ectendomycorrhizae of *Pinus patula* with *Amanita muscaria*. **1.** Unbranched mycorrhizal roots showing autofluorescence of the fungus mantle (× 40). **2.** Trans-sectional view of the mycorrhizal root with outer fungus mantle (FM) (× 120).

on the same medium on petri plates. Mycelial plugs of 0.25 cm^2 cut from the growing margin of a 21-day-old colony of the fungus were transferred to sterilized 250 ml Erlenmeyer flasks, each containing glass beads and 100 ml of Melin-Norkrans' solution[9] 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[10]. Each flask was inoculated with a single seedling. The seedlings were further grown in a growth chamber at 20 ± 1°C with 16 h photoperiod with light at 1000 lux. After two months of growth, the seedlings were inoculated with mycelial suspension of *A. muscaria*, 10 ml per flask. At the same time, 20 ml of Melin-Norkrans' solution[9] were added aseptically into each flask.

Two months after inoculation, the seedlings were removed from the flasks, their roots cleaned of adhering debris with water and examined for the presence of mycorrhiza by using a binocular zoom stereoscopic microscope. Mycorrhizal formation was observed in the roots which were fixed in formalin-propiono-alcohol, embedded in paraffin, microtomed and 10 μm thick sections were double stained with safranin-haematoxylin following the method of Johansen[11].

In vitro growth of *P. patula* seedlings inoculated with *A. muscaria* resulted in the emergence of several short lateral mycorrhizal roots from the mother root and each was covered with a mantle of hyphae. The short lateral mycorrhizal roots were simple unbranched type showing autofluorescence of their fungal mantle (figure 1). The transverse section of the mycorrhizal root showed characteristics of ectendomycorrhizae[12] such as sparse thin mantle over the crumpled epidermis (figure 2), presence of not-so-well-defined Hartignet in the intercellular spaces of cortical cells and intracellular penetration by hyphae in the cortical cells.

Pure culture synthesis of ectomycorrhizae using *A. muscaria* has been performed earlier in pines like *Pinus ponderosa*[12] and *P. sylvestris*[13]. There seems to be no report on the *in vitro* synthesis of ectendomycorrhizae of *P. patula* using *A. muscaria*.

Melin[14] and Norkrans[15] suggested that intracellular penetrations might be effected by fungi-forming mycorrhizae which could produce cellulase. Clowes[16] suggested that the mycorrhizal fungus could produce cellulase even when growing in symbiotic state also. The ability of our isolate of *A. muscaria* to produce cellulase *in vitro* has been reported earlier[17] and the ectendomycorrhizal formation in this case might be due to that ability in symbiotic state also.

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A REPORT ON SEED POLYMORPHISM IN *CROTALARIA BURHIA* BUCH.-HAM. IN INDIAN DESERT

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THE prevailing conditions in desert are not congenial for plant survival. Among various methods of adaptations, seed polymorphism is a common feature in plants of arid zone[1]. This includes production of viable seeds different in size, shape, weight, seed coat pattern, dormancy and germination requirement[2]. The variability in size and weight, which is genetically controlled, is often influenced by food during embryo development and seed maturation accentuated by the prevailing environmental conditions.

Crotalaria burhia is a characteristic leguminous species of sand dunes and sandy plains of Indian desert. It is a small bushy plant with bunches of erect stiff branches, equipped with deep root system, dense pubescent leaves and flattened stem during dry periods. Prakash and Sen[3] reported two forms in *C. burhia* based on distinct morphological variations as erect-bushy (EB) and suberect-spreading (SS). The present investigation reports the occurrence of adaptive polymorphism in the seeds of EB form in *C. burhia*.

Seeds from individual plants were collected separately and stored in polythene containers in laboratory. Germination experiments were conducted in continuous light. Since seeds possess hard seed coat, acid scarification for different durations was provided to remove the seedcoat[4].

Based on the distinct colour variation, the seeds were separated into four categories: A-yellow, B-brown, C-greyish black, and D-black (figure 1). The shape of the seeds ranged from round, semilunar to compressed. There is a clear notch near the hilum. The seeds of categories C and D are larger in size but lighter in weight, when compared with categories A and B. There was not much variation in seedling vigour and in the size of 1st cotyledonary leaf (table 1).

The four categories of seeds displayed primary dormancy, and did not show any germination in control. Pretreatment with concentrated sulphuric acid for 60 min gave optimum percentage of seed germination in categories A and D, whereas in categories B and C, it was in 40 and 10 min respectively. The germination percentage declined in seeds of categories B and C, when duration of acid pretreatment was increased. Clearly, seeds of categories A and D possess harder seed coat than B and C (table 2).

The arid zone seeds possess bizarre strategies for better establishment of plant species[4,5]. Probably arid zone is the only habitat where seeds are

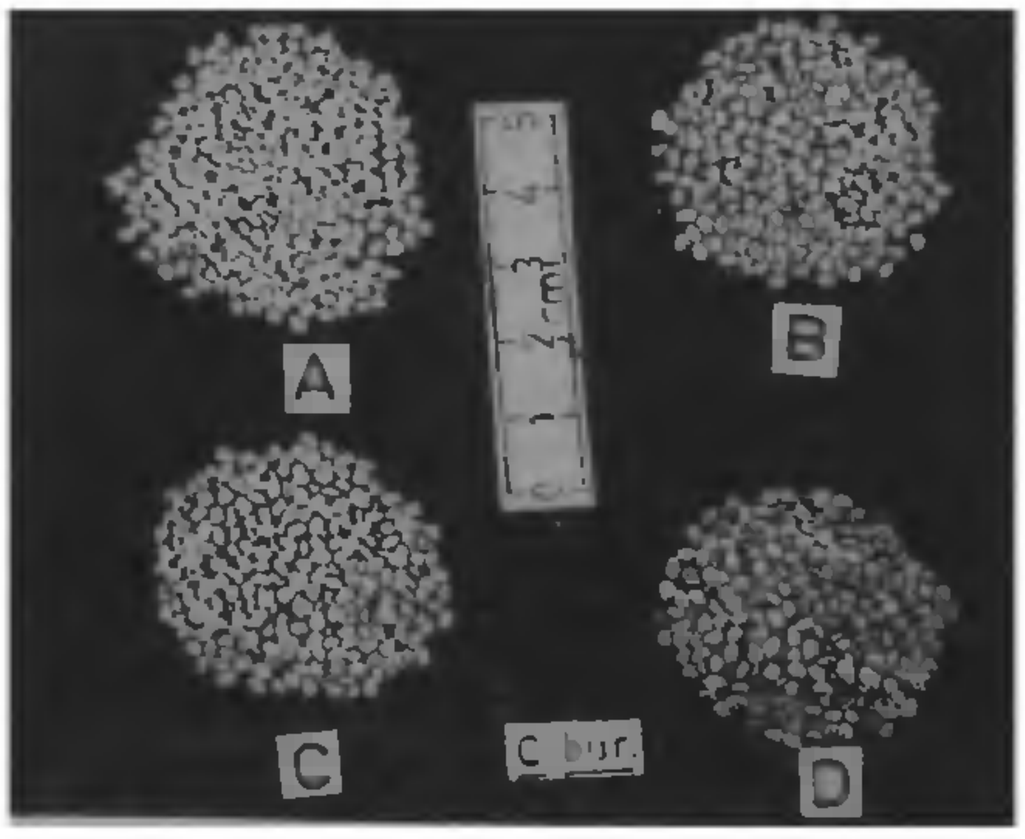

Figure 1. Four categories of seeds in *C. burhia*; yellow (A), brown (B), greyish black (C), and black (D).