

PINIRHIZA ALBA, A NEW ECTOMYCORRHIZAL TYPE ASSOCIATED WITH *PINUS ROXBURGHII* FROM PAKISTAN

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Abstract

Pinirhiza alba, is described as a new ectomycorrhiza associated with *Pinus roxburghii* from Pakistan. The mycorrhiza is characterized by milky white mycorrhizal systems, dichotomous branching pattern, thick plectenchymatous to pseudoparenchymatous mantle and loosely woven hyphae of uniform diameter forming rhizomorph. As so far no fungal partner of this ectomycorrhiza has been identified, hence it falls under the category of "unknown" mycorrhizae.

Introduction

Pines grow as wild plants in the mountainous regions of Pakistan but chir pine (*Pinus roxburghii*, Sargent) is cultivated and well adapted to the lower elevations in Lahore, Multan, Peshawar etc.

Tree of family Pinaceae (Pine, Fir, Larch, Spruce and Hemlock) have been reported ectomycorrhizal (Marx, 1991). Many types of "identified" and "unidentified" ectomycorrhizae occur with pines. "Identified" ectomycorrhizae are assigned the same name as that of the mycobiont forming ectomycorrhiza whereas "unidentified" mycorrhizae are given binomials (Agerer, 1991a).

During the investigation of ectomycorrhizae of trees of the Punjab plains, as unique type of ectomycorrhiza of "unknown" affinity was isolated. This mycorrhiza occurred with the root systems of about 40 years old *P.roxburghii* trees growing in Botanical Gardens, Punjab University. The mycorrhiza seems to be un-described previously, hence it has been given a binomial name.

Materials and Methods

Isolation of Ectomycorrhiza

Soil cores of roots of *Pinus roxburghii* were dug out in 8 × 16 cm size

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blocks containing intact soil, brought to the laboratory, and kept in polythene boxes to avoid any damage to the mycorrhizae. These blocks were soaked in water to loosen the soil particles. Roots were made free of soil and organic particles using camel hair brush. These roots were washed under tap water. The mycorrhiza was characterized following Agerer (1991b). Free hand made sections of root were cut and mounted in Lactophenol in trypan blue. The isolated mycorrhizal root tips were surface sterilized. Hydrogen peroxide (30%) was taken in a sterilized glass tube and cut mycorrhizal root tips were immersed in it for 20-30 seconds. These surface sterilized roots were given several washings in sterilized distilled water. Finally mycorrhizal root tips were washed in distilled sterilized water containing $50 \mu\text{g ml}^{-1}$ streptomycin and $20 \mu\text{g ml}^{-1}$ tetracycline to control bacteria. These surface sterilized mycorrhizal root tips were transferred aseptically to MMN medium in McCartney bottles. These bottles were incubated at $\pm 2^\circ\text{C}$ in dark.

The isolated mycorrhizal root systems and their cross-sections were photographed. The culture isolates on the MMN medium were also characterized.

Results

Morphological characters (Plate 1, Fig.a-c)

The young ectomycorrhizae were milky white when collected whereas old mycorrhizae were slightly darker due to brown coloured roots. Ramifications were dichotomously branched and dichotomous united to form a semi-rosetts structures upto 3 cm in diameter. The surface of the dictomous branching was shiny. Ectomycorrhizal systems were 7 mm - 1 cm in length and 2.0 mm in thickness. Un-ramified ends were upto 0.5 mm and axes 0.3 - 0.5 mm at extreme tips. Rhizomorphs were not abundant, white, flat. Surface of the rhizomorph was smooth. They were approximately 0.2 mm thick.

Anatomical Characters

Anatomical features of the mantle in plan view (Fig.1 f,g)

In plan view, the mantle surface was found to be transitional type between plectenchymatous and pseudoparenchymatous (type A in Agerer, 1991b) ring like with some short cells inbetween. Perpendicular hyphae were larger than those which run parallel and grew over mantle surface in irregular manner. The mantle

surface possessed yellowish crystals. Hyphae were 3.0 - 3.7 μm diameter in width, thin-walled and septate. Septa were simple and clamps were not seen.

Innermost layer of the mantle (Fig.2 h,i)

Innermost layer of the mantle was more or less similar to the outer surface of the mantle but hyphal growth near or on the surface of the mantle was more. The hyphae were more in number than those of the surface. Hyphae were septate and 2-4 μm in diameter, and no clamps were seen. Hyphae protruding perpendicular to the surface were roundish.

Rhizomorphs (Fig.2 b,c)

Rhizomorphs consisted of loosely woven hyphae of uniform diameter. These were undifferentiated, of type A/B (Agerer, 1991b). Some hyphae were with rough surface (spiny walls) but most of the hyphae were with smooth walls. The hyphae forming the rhizomorphs were with frequent clamps, however, a few were without clamps. Hyphae were 3.5 - 4.23 μm in diameter and inflated at clamps. Rhizomorph origin was variable but it concentrated at the tips of the mycorrhiza.

Colour reactions in different reagents

Fuchsin: No reaction (n.r.), aniline: wall bluish pink, Ethanol: n.r., KOH (1%): n.r., lactic acid, yellowish appearance, FeSO_4 : n.r.

Culture Characteristics

The MMN medium inoculated mycorrhizal root tips gave white mycelial growth after 15 days of inoculation. The isolated fungal colony growth reached upto 1.5 and 2 cm in diameter within 2 weeks and after 4 weeks respectively. The hyphae were hyaline, with clamps and septa.

Reference Specimen

Pakistan, Lahore, Botanical Garden, Quaid-e-Azam Campus, Punjab University with *Pinus roxburghii*, Nov. 15, 1996 in SHI Mycological Herbarium Punjab University, AN 786.

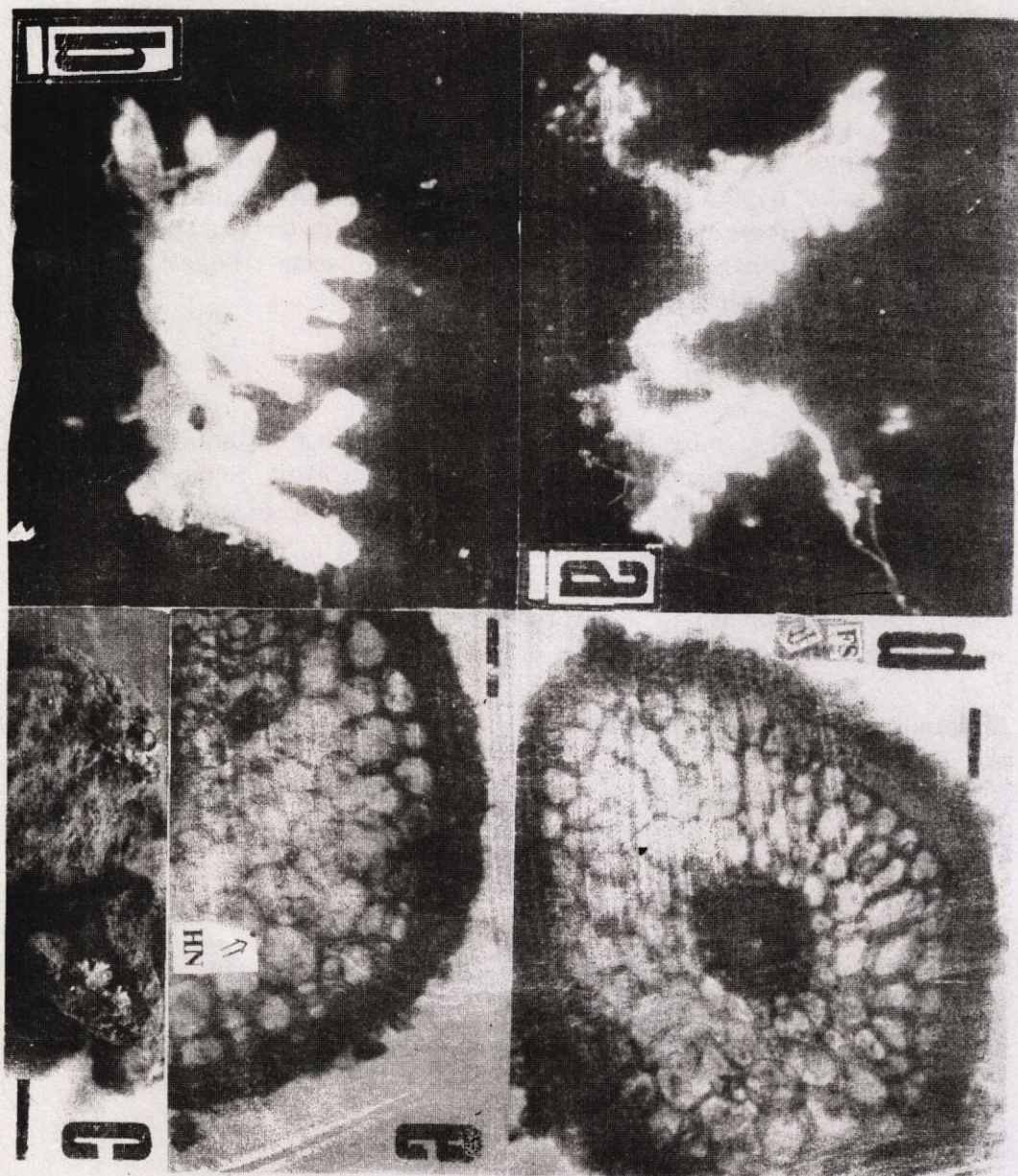


Plate a-e: *Pinirhiza alba*. a-b: Dichotomously branched white mycorrhizal system, c: Mycorrhizal system attached to the rhizosphere soil particles, d, e: Cross-section of ectomycorrhizal root showing fungal sheath and Hartignet.

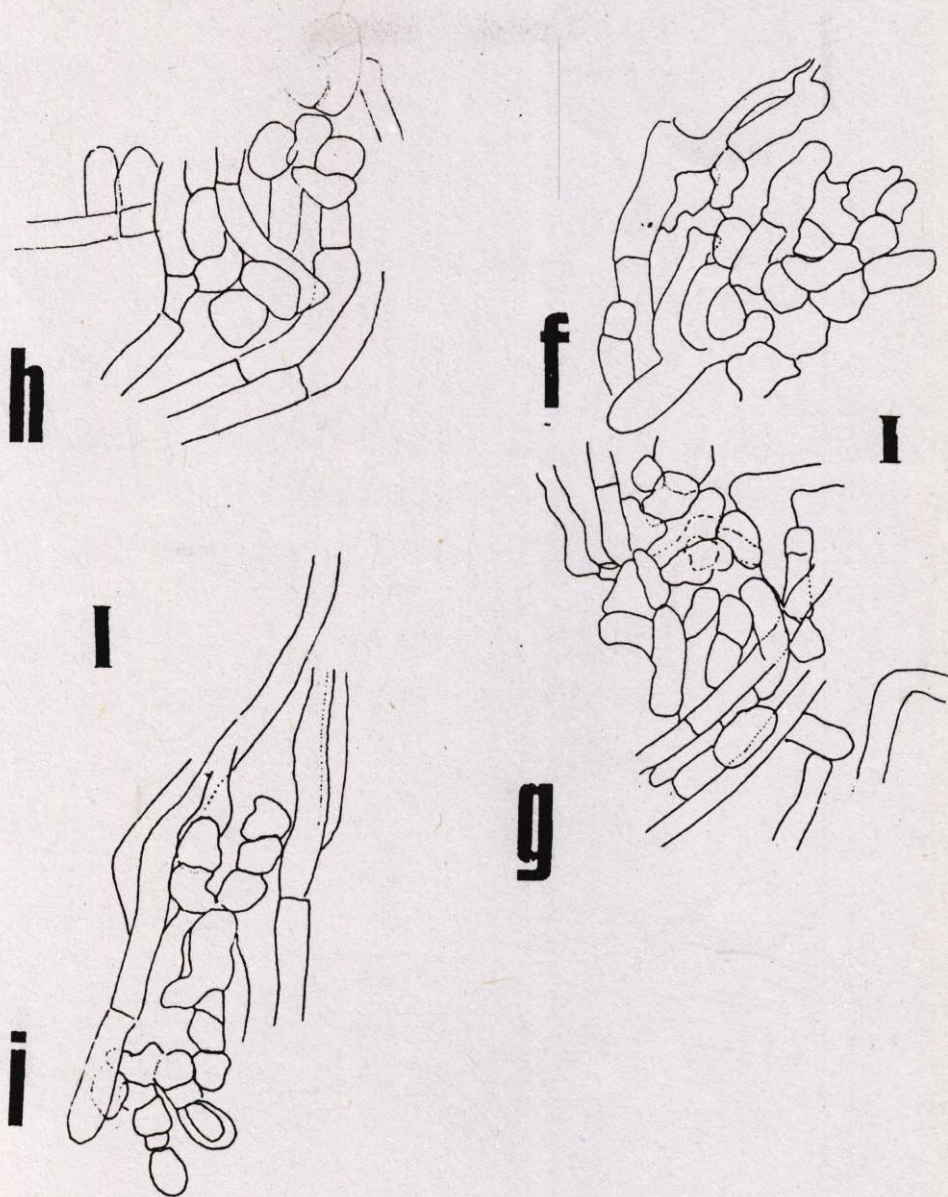


Fig.1.f-g: *Pinirhiza alba*. f, g: Plane view of outer mantle surface, h, i: Plane view of inner mantle surface.

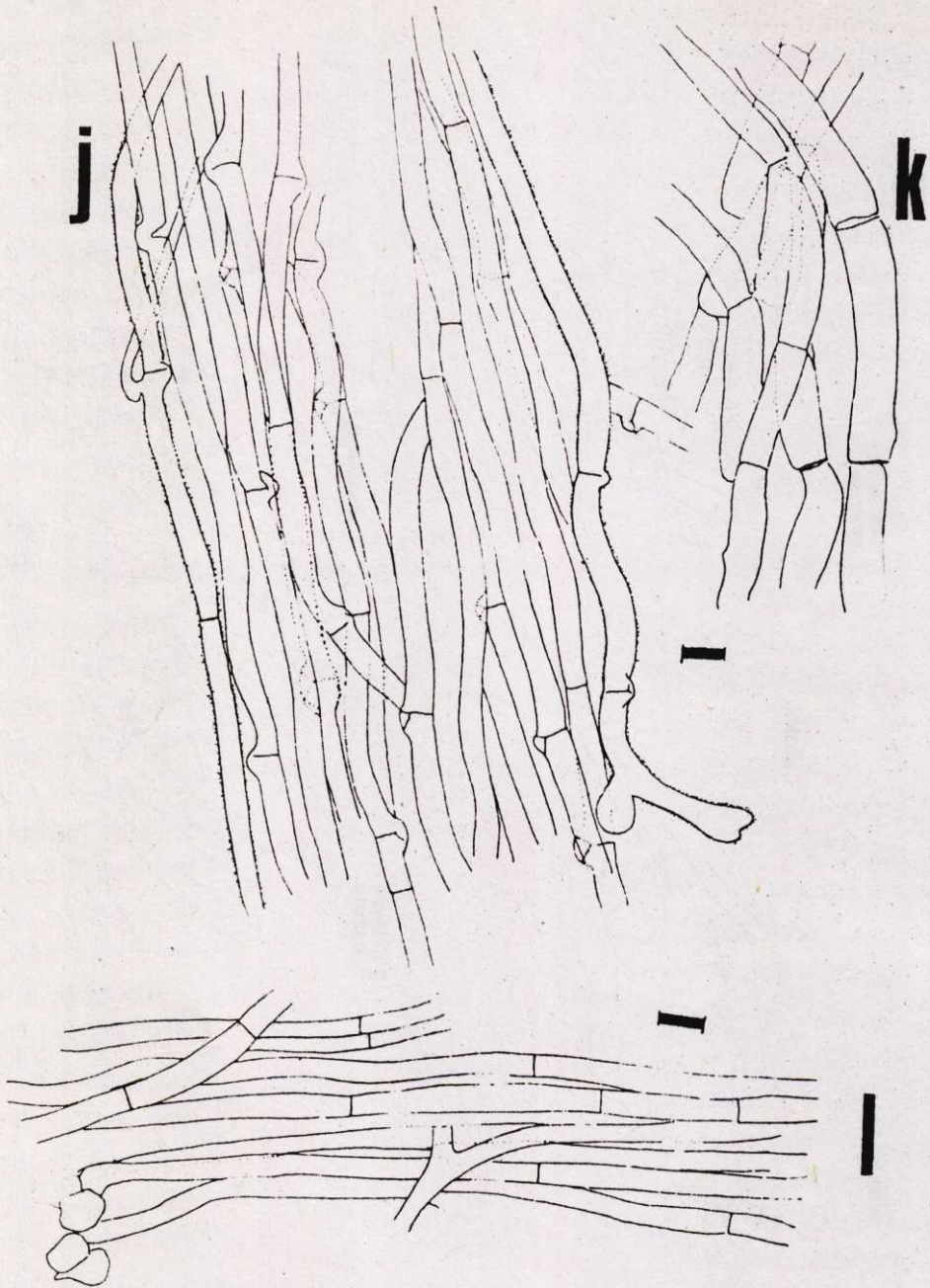


Fig.2 j-l: *Pinirhiza laba*. j, k: Rhizomorphs, l: Mantle peeled off.

Scale Bar: = 3 cm for a, b: 1 cm for c, 20 μ m for d, e, and 7 μ m for f-l

Discussion

The most striking features of the *Pinirhiza alba* are its milky white colour, dichotomous branching pattern, thick plectenchymatous to pseudoparenchymatous mantle and loosely woven hyphae of rhizomorphs.

Already "unknown" mycorrhizae reported include *Pinirhiza angularis*, *P. ochrolactans*, *P. rosea*, *P. spinulosa*, *P. subalpina* and *P. tortuosa* (Agerer, 1987-96). Our newly reported mycorrhiza *Pinirhiza alba* is closer to *P. subalpina* reported on *Pinus cembra* and *Pinirhiza tortuosa* found on roots of *P. sylvestris*. *Pinirhiza tortuosa* resembles with newly reported mycorrhiza due to its white colour. However, it can be distinguished from *P. tortuosa* due to its dichotomous branching pattern and from *P. subalpina* due to thick walled cystidia on the mantle surface. *Pinirhiza alba* has been reported on *Pinus roxburghii*.

Pinirhiza subalpina is characterized by its brownish colour with white tips. Mantle in the surface view is pseudoparenchymatous and bears thick walled cystidia.

In case of *P. alba*, the mycorrhiza as a whole is milky white and turns light brown later on. The mantle in the surface view is unlike *P. subalpina* between a plectenchymatous and pseudoparenchymatous pattern and no cystidia occur in this newly described mycorrhiza. *Pinirhiza subalpina* has been reported on *Pinus cembra* whereas the host tree for *P. alba* is *Pinus roxburghii*. It can be safely concluded that this mycorrhizal type is newly reported and falls in the category of "unknown" mycorrhizae.

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