

MYCORRHIZAL ASSOCIATIONS IN GYMNOSPERMS OF WEST PAKISTAN

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Abstract

Mycorrhizal associations in gymnosperms collected from West Pakistan have been investigated. All the gymnosperms examined are mycorrhizal. Ectotrophic association is found only in the members of the family Pinaceae, whereas rest of the Coniferales, members of orders Ginkgoales, Taxales and Ephedrales, are found to have endotrophic mycorrhizal associations. *Endogone* spores are present in the rhizospheres of endotrophic mycorrhizae. Detailed investigations regarding the types of spores and their ability to synthesize the vesicular-arbuscular mycorrhizae are in progress and their results will be made available on the conclusion of these experiments.

Introduction

The symbiotic associations of fungi with the roots of higher plants are well known. As early as 1885, Frank in Germany coined the name "mycorrhiza" for this association. The symbiosis is of mutual benefit to both the organisms and without this association both the plant and the fungus, under certain environmental conditions, suffer from nutritional deficiencies. Romell (1938) has demonstrated that if mycorrhizal fungi are not in association with trees, they are not capable of producing fruiting bodies. Recently obtained evidences indicate that mycorrhizal plants grow better than non-mycorrhizal ones in the soils deficit in phosphorus and nitrogen (Nicolson 1967).

Except for few studies, in Indo-Pakistan very little work has been done on the occurrence and distribution of mycorrhizae. Chaudhuri and Akhtar (1931) recorded the presence of endophytic fungus in the nodules of *Podocarpus chinensis*. Later in 1939, Mohan took a study of roots of *Pinus longifolia*, *P. excelsa*, *Picea morinda*, *Abies spectabilis*, *Cedrus deodara*, *Juniper* spp., and *Cupressus* spp. for the occurrence of mycorrhizae. Recently Anver and Khan (1959) made observations for mycorrhizae in *Pinus roxburghii*, *P. wallichiana* and *Cedrus deodara*.

Materials and Methods

Specimens of root material of gymnosperms were collected from Khanspur (Murree Hills) and from plants grown in the local botanical gardens. Roots from seedlings as well as trees were dug out and washed gently. They were fixed in F.A.A.

Fixed roots were then examined microscopically after clearing them by boiling in cotton-blue lactophenol mixture for about thirty minutes.

Hand sections, both transverse and longitudinal, were prepared using a razor-blade. They were stained with cotton-blue or Sudan IV in lactophenol. The stained sections were examined after mounting them in lactophenol.

Observations

Ectotrophic mycorrhizal associations: Out of 15 species of 12 genera examined, ectotrophic mycorrhizal associations were found in 5 species, namely, *Abies pindrow* Royle III, *Picea smithiana* (Wall) Boiss, *Cedrus deodara* (Roxb. ex Lamb.) G. Don, *Pinus wallichiana*, A.B. Jackson, and *P. roxburghii* Sargent, all members of the family Pinaceae, while the rest possessed endotrophic mycorrhizal associations.

The specimens of all these species, except few which were cultivated in Lahore, were tall trees growing in the Western Himalayas. All the fine rootlets were covered with a fungal mantle of fine septate hyphae. In the case of *P. wallichiana*, *A. pindrow* and *C. deodara*, young seedlings growing beneath the parent trees, were also examined. There was an extensive covering of ectotrophic fungal hyphae on the fine roots of these seedlings. The fungal hyphae were fine, colourless, septate and about $0.5-1\mu$ in diameter. The mantle was $20-25\mu$ thick. The mantles, on the roots of tall trees, however, were thicker and ranged from $30-35\mu$ in diameter. The root-cap of most of the specimens were entirely covered with the mantle.

In the case of *P. wallichiana*, *A. pindrow*, *C. deodara* and *Picea smithiana* the fruiting bodies of basidiomycetous fungi such as *Agaricus* spp. and *Lycoperdon* spp. were found growing around the base of these trees. The fungal hyphae around the roots of these plants were found in direct connections with the fruiting bodies of agarics and puff-balls and they bore prominent clamp connections.

Pinus species showed the usual heterorrhizic root systems composed of long and short roots (Wilcox 1964). The short roots were dichotomously branched and had a 'Hartig net' surrounding the epidermal and outer cortical cells that were radially elongated (Fig. 1). The long roots had an external fungal sheath without a Hartig net. The ectotrophic mycorrhizae lacked root hairs.

Endotrophic mycorrhizal associations: Roots of *Ginkgo biloba* L., *Taxus wallichii* zucc., *Araucaria cunninghamii* Aiton ex D. Don, *Cupressus sempervirens* L., *C. torulosa* D. Don, *C. funebris* Endl., *Thuja orientalis* L., *Juniperus communis* L., *Taxodium mucronatum* Ten., and *Ephedra ciliata* Fisch and Mey ex Boiss, showed fungal hyphae within their tissues.

In morphology these endotrophic mycorrhizae resembled closely with the most widespread and universal vesicular-arbuscular mycorrhizae (Nicolson 1967). Examination of the whole roots revealed the presence of external mycelium on the surface of the roots of most of the species (Fig. 2). The external hyphae were thick walled, non-septate, deeply stained and about 15-20 μ in diameter. No dimorphic characteristics of the mycelium were observed nor any spores were found attached to the external mycelium.

The root hairs were present on the endotrophic mycorrhizae as apposed to the ectotrophic mycorrhizae. The infection of roots through the root hairs was not observed. Hyphae were seen penetrating the epidermis. Inside the cortical cells the hyphae were coiled and almost filling the cells completely (Fig. 3). While passing through the cell wall the inter- and intracellular hyphae remained unstricted.

Two fungal structures, arbuscules and vesicles, the diagnostic features of the endophyte, were observed in the root cortex. In structure these features were similar to the ones described by Janse (1896), who first discovered and named these structures. In general, arbuscules were formed in the inner cortex. These consisted of dichotomously branched intercellular hyphae, the distal branches of which appeared to disintegrate soon after they are formed, giving the invaded cell a granular appearance (Fig. 4). Vesicles developed in the outer cortex as terminal swellings of the fungal hyphae (Fig. 5). These were thick walled and approximately 30-40 μ in diameter. Arbuscules were seen in all the specimens examined, whereas the vesicles were observed only in the roots of trees alone. The nucleus in invaded cells was found much enlarged.

The fungal endophyte in the vesicular arbuscular mycorrhizae has been placed in Phycmycetous genera like *Rhizophagus*, *Pythium* or *Endogone* (cf. Hawker 1962). The reason for this discrepancy in the literature is due to the difficulty in isolating the endophyte in pure culture. There is no way of testing this other than by visual evidences of the presence of spore-like bodies in the rhizosphere, which are capable of synthesizing vesicular arbuscular mycorrhizae. Spores or fruit-bodies similar to those of *Endogone* described by Mosse (1953) and extracted from the soil by Gerdemann and Nicolson (1962, 1963), were found in rhizospheres of all the species examined. Many different types of thick walled *Endogone* spores (Fig. 6) were collected from the rhizospheres by the wet-sieving and decanting technique described by Gerdemann and Nicolson (1963).

Discussion

All the gymnosperms examined show mycorrhizal associations. All the species found to develop ectotrophic mycorrhizal associations were members of the family Pinaceae, whereas the members of the families Araucariaceae, Cupressaceae, Taxodiaceae and of orders Ginkgoales, Taxales, and Ephedrales, contained vesicular-arbuscular endophyte. Mohan (1939) recorded the presence of ectotrophic mycorrhizae in *Pinus* spp. and *Picea morinda*, ect-endotrophic mycorrhizae in *Cedrus deodara*, and endotrophic mycorrhizae in *Abies spectabilis*, and their absence in *Juniperus* and *Cupressus* spp. According to Anvery and Khan (1959), all the three species studied by them possessed both ectotrophic and endotrophic mycorrhizae on the sublateral rootlets. From the present study, however, it appears that there is a clear pattern with regard to the occurrence of mycorrhizae amongst the Ginkgoales, Taxales, Coniferales and Ephedrales. Endotrophic mycorrhizae seem to be of general occurrence in all the families except Pinaceae, the members of which form the ectotrophic type, though sporadic ectotrophic mycorrhizae may occur elsewhere—as in *Juniperus communis* where *Cenococcum* forms ectotrophic mycorrhizal association instead of its usual endophytic one (Lihnell 1939). These observations are consistent with those of earlier workers (Noelle 1910; Laing 1923) and with those of author's findings in Australia, (Khan 1968).

In most of the specimens possessing ectotrophic mycorrhizae, the root apex was entirely covered with the mantle, as observed by Redhead (1968) in the case of *Azelia africana*. Clowes (1954), however, has shown that mantle in *Fagus sylvatica* roots does not cover the root apex. The Hartig net was observed on the short roots only while long roots lacked it. Robertson (1954), however, had made an observation contrasting to this and he observed a Hartig net on the long roots of *Pinus sylvestris*. The observations that ectotrophic mycorrhizae had no root hairs are in accord with those of Morrison (1960) who found that the ectotrophic mycorrhizae of *Nothofagus menziesii* lacked root hairs.

The presence of direct connections of fungal hyphae of agarics and puff-balls with the ectotrophic mycorrhizae indicate the possibility that these ectotrophic mycorrhizae belong to the basidiomycetous fungi. This is in good agreement with the conclusions of Melin and his associates (for full reference of these workers see Trappe 1962).

Unlike ectotrophic mycorrhizae, the distinction between mycorrhizal and non-mycorrhizal roots of endotrophic mycorrhizae was not possible as there were

no morphological or colour differences visible to the naked eye. Jones (1924) and Gerdemann (1961), however, observed that mycorrhizal roots of peas and maize, respectively, are yellow in colour as compared to non-mycorrhizal roots. Roots of *Araucaria cunninghamii* bore elongated nodules analogous to those occurring in *Podocarpus* (Khan 1967), but they are not caused by the fungal associations but are the constant morphological features in Araucariaceae (Khan 1968).

The fungus observed on the surface of the endotrophic mycorrhizae do not form a compact sheath or mantle of mycelium like that of ectotrophic mycorrhizae. Nicolson (1959), Greenall (1963) and Khan (1968) observed dimorphic nature of the external mycelium on the surface of endotrophic mycorrhizae of grasses, *Griselinia littoralis* and *Podocarpus lawrencei*, respectively. Butler (1939) Nicolson (1959), and Khan (1968) recorded the occurrence of extramatrical chlamydo-spores on the hyphae attached to the root. No doubt the dimorphic characteristics of mycelium and the external spores, found to be absent in this survey, would be found to be present under other conditions were a more extensive search made.

As observed by Redhead (1968) in *Khaya invorensis* and by Greenall (1963) in *Griselinia littoralis*, the endophytic hyphae remained unconstricted while passing the cell walls. The endophyte did not penetrate into or beyond the endodermis and was found strictly limited to the cortex. The endodermis appeared to form a "tannin barrier" similar to that observed by Clowes (1951) in the ectotrophic mycorrhizae of *Fagus sylvatica*. The nucleus in invaded cells was found much enlarged but no pleurinnuclear condition was observed as found by Shibata (1902) and Khan (1968) in *Podocarpus* spp. and by Lihnell (1939) in *Juniperus communis*.

The absence of vesicles in the roots of seedlings is in accord with the observations of Mosse (1963) who found that the vesicles, in infected clover seedlings, appeared first in the oldest parts and the production of vesicles increased greatly with the increase in age.

Most of the spores extracted from the rhizospheres of endotrophic mycorrhizae fitted the description of one or the other of the eight types found in the Australian and New Zealand soils by Mosse and Bowen (1968). Few new types of spores were also discovered, a detailed account of their structure and ability to produce vesicular—arbuscular mycorrhizae will be published latter.

There are many reports of evidences showing that the mycorrhizal associations—ectotrophic as well as endotrophic—are beneficial or even essential to tree growth (cf. Harley 1963; Mosse 1963).

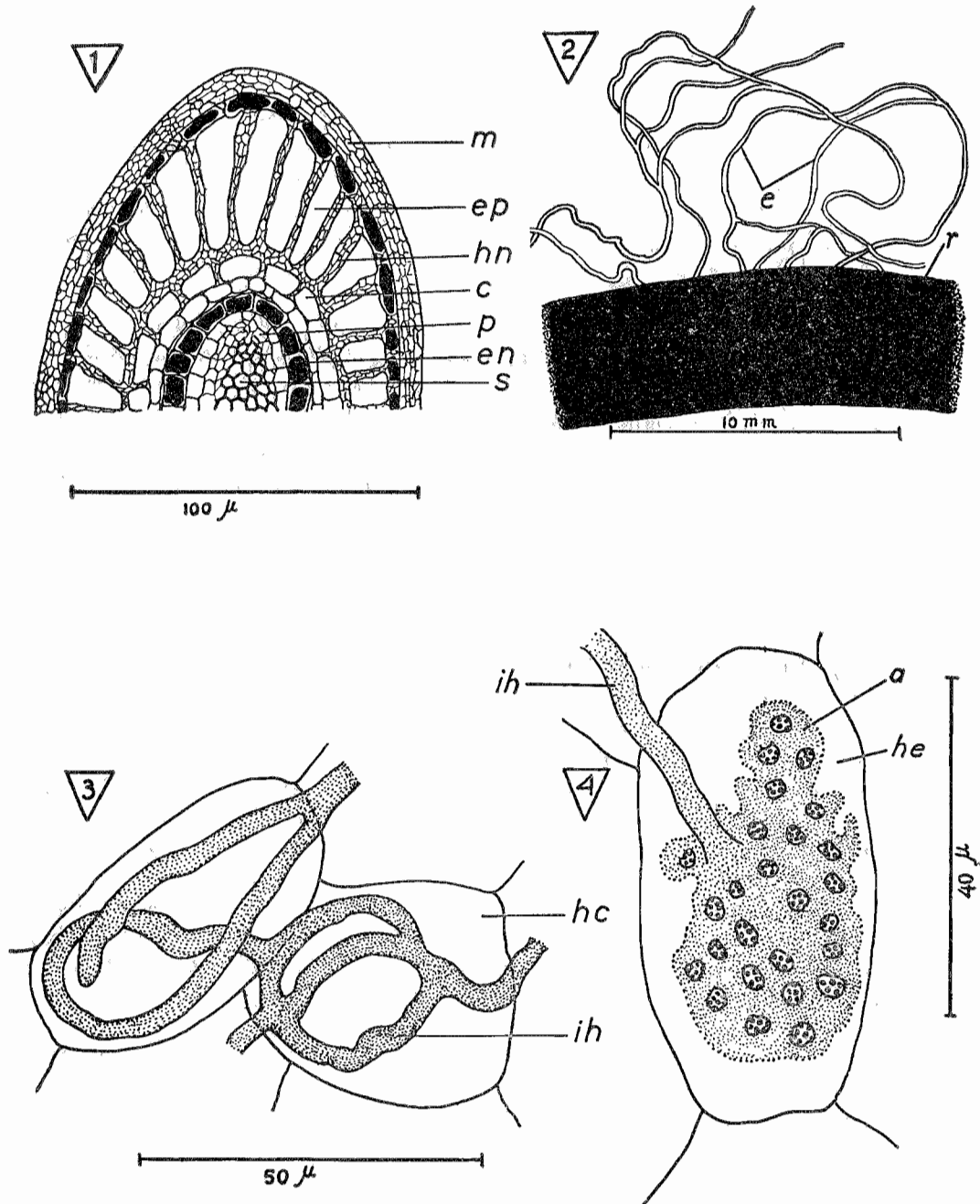


Fig. 1. T.S. of short root of *Pinus wallichiana*; c, cortex; en, endodermis; ep, epidermis; hn, Hartig net; m, mantle; p, pericycle; s, stele.

Fig. 2. Root of *Taxus wallichii* showing external mycelium (e) on the root surface (r).

Fig. 3. Cortical cells (hc) of mycorrhizal root of *T. wallichii* showing the intracellular fungal hyphae (ih) filling the cell lumen.

Fig. 4. Cortical cell (hc) of mycorrhizal root of *T. wallichii* showing disintegrated arbuscule (a) at the tip of an intracellular hypha (ih).

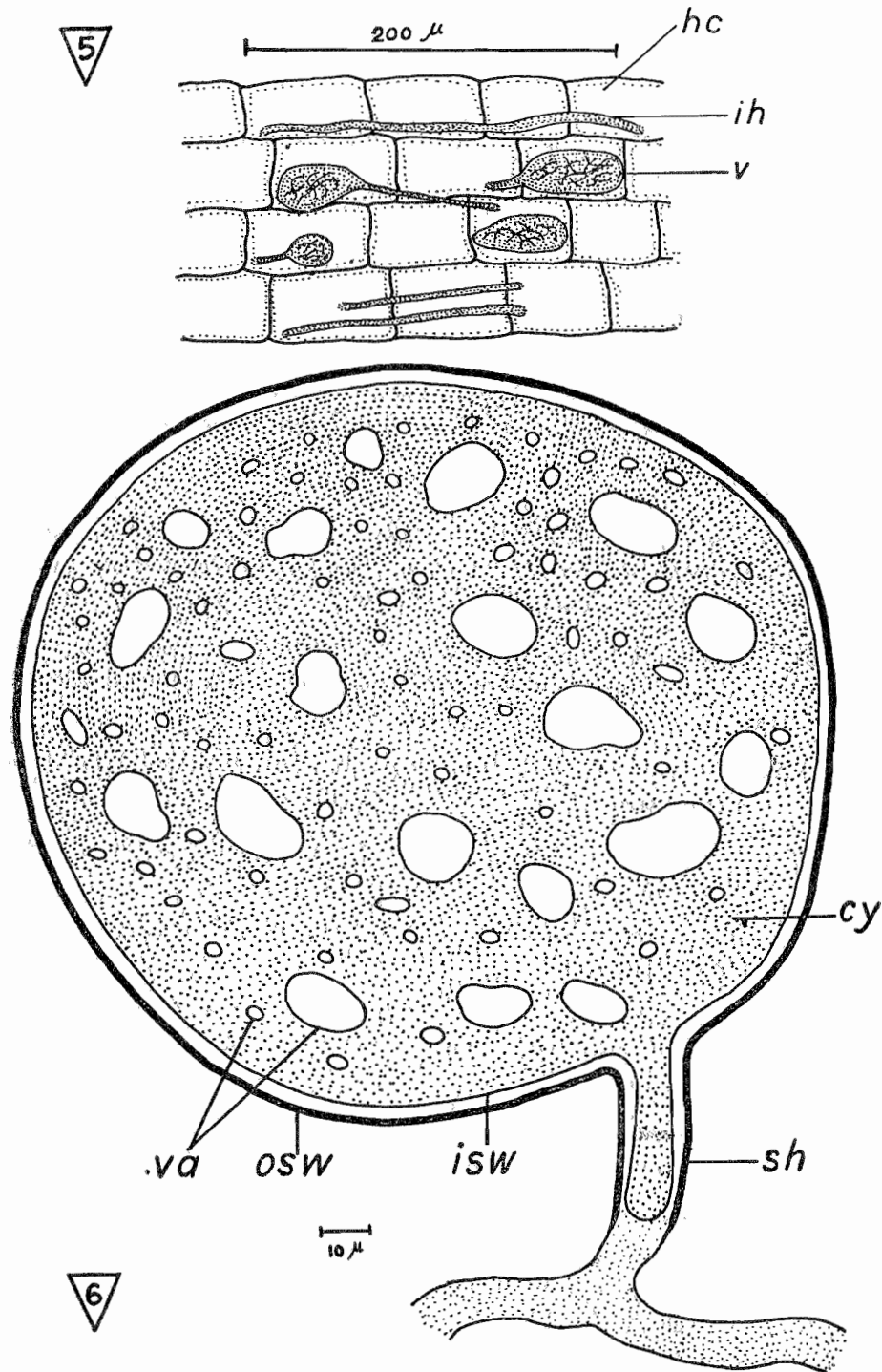


Fig. 5. L.S. of mycorrhizal root of *T. wallichii* with vesicles; hc, host cortex; ih, intracellular hypha; v, vesicle.

Fig. 6. Yellow vacuolated type of *Endogone* spore extracted from the rhizosphere of *T. wallichii*; cy, cytoplasm; isw, inner spore wall; osw, outer spore wall; va, vacuoles.

The present study in West Pakistan confirms the almost universal occurrence of mycorrhizal association in gymnosperms. Further investigations are on way to ascertain the nature of the fungi—especially those forming vesicular arbuscular associations, and the effects of these associations on the growth of trees under tropical climate of West Pakistan.

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