SOME OBSERVATIONS ON MYCORRHIZAE OF *OLEA CUSPIDATA* WALL.

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Abstract

Mycorrhizae were found on the specimens of *Olea cuspidata* Wall., collected from the scrub forest in the Salt Range west of Jhelum in the foot hills of the Himalayas. Mycorrhizal root system was heterorrhizic. Both ectomycorrhizae and endomycorrhizae were found on the same plant but on different roots. Endomycorrhizae were covered with a 20-30 µ thick, parenchymatous and smooth mantle of fungus. No hartig net was observed. In endomycorrhizae the cortex contained an abundance of thick walled vesicles and coils of aseptate hyphae, characteristics of Vesicular Arbuscular Mycorrhizae caused by *Endogone fasciculata*. No *Endogone* spores were recovered from soil around olive roots by the wet sieving and decanting technique because of the extremely smaller size, always under 100 µ, that they would not be retained on the sieves. No attempts were made to isolate the causal fungi. Significance of these findings in the cultivation of Olive in Pakistan is discussed.

Introduction

During the last few years it has become evident that under natural conditions a phycomycetous fungus *Endogone* has developed a non-pathological and mutualistic relationship with roots of most higher plants and that non-mycorrhizal plant is more the exception than the rule (Nicolson, 1967; Gerdemann, 1968; Khan, 1972, 1973). This ubiquitous occurrence of mycorrhiza is due to the presence of many different types of *Endogone* spores as regular and universal components of biological communities found in natural soil habitats (Mosse & Bowen, 1968; Khan, 1971; Sutton & Barron, 1972). Most probably the plants known to be non-mycorrhizal would be found to be mycorrhizal under other conditions were an extensive search made or there might be some fungitoxicant substance(s) associated with their defence mechanism against fungal attack.

The genus *Olea cuspidata* is generally non-mycorrhizal and no *Endogone* spores were recovered from its rhizosphere (Khan, 1973) though mycorrhiza has been found on a few specimens collected this year from the scrub forests in the salt range west of Jhelum in the foot hills of Himalaya. The present paper deals with the preliminary observations on the mycorrhizae of *Olea cuspidata* Wall.

Materials and Methods

Specimens of root material were collected from medium sized evergreen trees. Roots were fixed in formalin-acetic acid-ethanol. Hand sections, both longitudinal and transverse were prepared and stained with trypan blue in lactophenol.

The extent of mycorrhizal infection was measured by recording the percentage of root pieces infected after clearing 1 cm pieces for 30 minutes in potassium hydroxide (10% at 90°C) and staining with 0.05% trypan blue in lactophenol in a manner described by Phillips & Hayman (1970).

Soil around roots of *O. cuspidata* was wet sieved for the extraction of *Endogone* spores previously described (Khan, 1971).
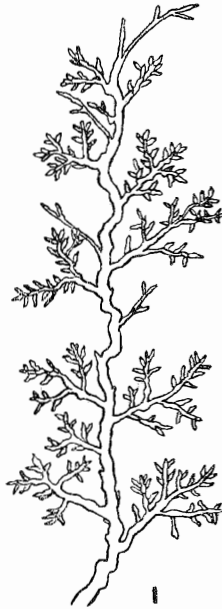
Fig. 1.   Line drawing of a root of *Olea cuspidata* bearing abundantly branched and thicker mycorrhizal and less branched and thinner non-mycorrhizal laterals.

Observations

A specimen taken from a somewhat isolated tree near the Choa Saiden Shah Civil Rest House proved to be non-mycorrhizal.   Other specimens collected from scrub forests in the salt range, consisting principally of *Olea cuspidata*, *Acacia modesta* and *Monotheca buxifolia* were partially mycorrhizal.

The mycorrhizal root system was very characteristic.   There was a differentiation of laterals into 'long' and 'short' roots.   The 'long' roots are potentially capable of indefinite growth in length whereas the 'short' roots are of restricted growth in length and are abundantly branched (Fig. 1).   The mycorrhizae were numerous and at first sight the bearing root looked very much similar to beech mycorrhizae.   In contrast, the non-mycorrhizal roots, inter-spersed with mycorrhizal ones, as only a portion of laterals developed into mycorrhizae, were less branched and thinner.

All the mycorrhizal short roots were covered with a fungal mantle of fine, septate, colourless and about 1 μ diameter hyphae.   The mantle was mostly parenchymatous, 20-30 μ thick and smooth on the outside with one or two filaments extending out into the soil from the outermost layer (Fig. 2).   The root caps of most of the specimens were covered with the mantle.   The ectomycorrhiza lacked root hairs whereas the un-infected laterals, inter-spersed with mycorrhizae, bore root hairs.   The epidermal cells were elongated radially on one side of the root only but no Harting network of hyphae between these cells was found (Fig. 2).
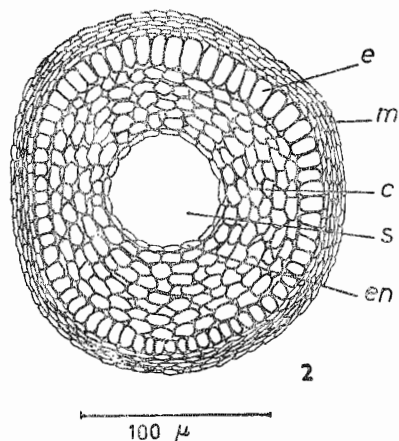
Fig. 2. T.S. of ectomycorrhiza of *O. cuspidata*. Note radial elongation of epidermal cells on one side of the root. c, cortex; e, epidermis; en, endodermis; m, fungal mantle; s, stele.
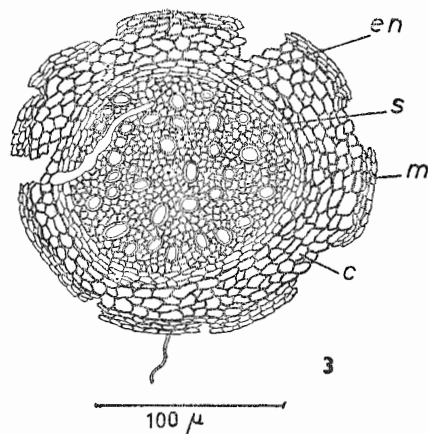
Fig. 3. T.S. 'long' root of *O. cuspidata* showing mantle covering. Note the absence of Hartig net. c, cortex; en, endodermis; m, fungal mantle; s, stele.

The long roots of olive were also occasionally found to be covered by a thin, filamentous but compact and smooth mantle (Fig. 3). No extension of hyphae between cortical or epidermal cells in the form of Hartig net was observed.

In *O. cuspidata* both ectomycorrhizae and vesicular arbuscular endomycorrhizae occurred on the same plant but on separate roots. In endomycorrhizae the cortex contained coils of aseptate fungal hyphae filling the cell lumens and an abundance of thick walled vesicles, damaging the cortical cells (Figs. 4 and 5). The extent of vesicular infection was fiarly high as more than 90% of root segments examined were infected.

No *Endogone* spores were recovered from soil around olive roots by the wet sieving and decanting technique.

Discussion

While the mechanisms regulating mycorrhiza development are undoubtedly complex, imperfectly understood and require much more detailed investigation, it is generally considered that even in those closely related groups of plants reported to be non-mycorrhizal, some plants are liable to become involved in symbiosis, totally or partially because of environmental or other conditions. This is borne out by the present study and a previous survey by Khan (1973).

Both ectomycorrhiza and endmoycorrhizae were found on the same plant of *Olea cuspidata*. Although the occurrence of the two types on the same species is apparently rare, it is not unknown, since previous workers (McDougall & Jacobs, 1927; Baylis, 1962; Shuja, Gilani & Khan, 1971) reported such cases. Such cases have also been reported to occur in members of families Salicaceae, Juglandaceae, Tilliaceae and
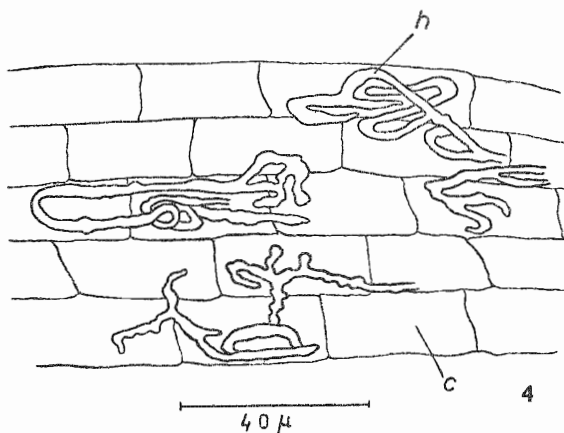
Fig. 4.   Cortical cells (c) of endomycorrhiza of *O. cuspidata* filled with intracellular coils of fungal hyphae (h).
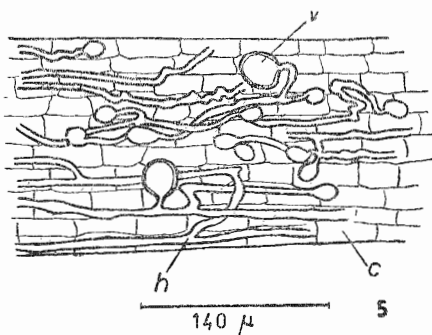

Fig. 5.   Cortical cells (c) of endomycorrhiza of *O. cuspidata* containing many thick-walled vesicles (v) and fungal hyphae (h).

Myrtaceae which are either ectomycorrhizal or endomycorrhizal, or both types may occur on the same plant (Gerdemann, 1968). Probably more extensive research will increase the known number of species with both types of mycorrhizae on the same plant.

Unilateral radial elongation of epidermal cells of ectomycorrhizae of *O. cuspidata* is in agreement with the observations of McDougall & Jacobs (1927) who noted a similar morphological phenomenon in *Populus tremuloides*. However, such cases have never been reported for ectomycorrhizae of conifers.

No attempts were made to isolate or identify the fungi causing mycorrhizae. The endophyte causing endomycorrhiza in olive is probably *Endogone fasciculata* as it is characterised by an abundance of thick walled vesicles in root cortical cells. The failure to recover the spores of the endophyte from the soil around olive roots is because

of their smaller size, always under 100 μ in diameter, that they would not be retained on the sieves (Gerdemann, 1955, 1969). Such cases, for instance, are reported by Nicolson (1960) who noted high endophyte activity but no *Endogone* spores in sand dunes. Khan (1973) also recorded that despite high percentage of root segments of *Salvadora oleoides, Acacia senegal, Gymnosporia montana* and *Impomaea biloba* containing a well developed vesicular arbuscular infection in their cortices, very few spores have been recovered from their rhizospheres. *Endogone fasciculata* is probably involved in such cases. A floatation-adhesion technique, recently described by Sutton & Barron (1972), may be a useful technique for the recovery of *Endogone* spores which are less than 100μ in diameter.

Plants benefit from mycorrhizal associations due to an intervention of mycorrhizal, fungi in increasing solubility of minerals, improving uptake of water and nutrients, especially phosphorus, for host plants, protecting host roots against pathogens and producing plant growth hormones. Without associations with specific mycorrhizal fungi, most plants, including many forest and horticulturally important species, could not survive in natural soils. These findings are potentially of great practical value in afforestation and in increasing food production in those areas of the world where phosphorus limits growth and phosphorus fertilization is not economical. Mycorrhizal inoculation of plants with *Endogone*, like inoculation of legumes with nodule bacteria and forest trees with ectomycorrhizal fungi, to promote growth, merits further consideration, especially for plants like olive that are usually transplanted. This method can be utilized in cultivation of Olive (*Olea europaea*), the fruits of which yield the most important of the non-drying food oils, in Pakistan. Mycorrhizal inoculation of cuttings by growing them in containers or seed-beds with high population of *Endogone* spores of an effective strain or species, would be of practical value in olive cultivation.

Acknowledgements

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