VESICULAR-ARBUSCULAR MYCORRHIZAE IN PLANTS AND ENDOGONACEOUS SPORES IN THE SOIL OF NORTHERN AREAS OF PAKISTAN

III-dir and Chitral

S. R. SAIF, I. ALI AND A. A. ZAIDI

Department of Biological Sciences, University of Islamabad.

Abstract

The incidence of vesicular-arbruscular (VA) mycorrhizae in roots and spores of species of Endogonaceae in soil surrounding the roots of 200 plants belonging to 47 families collected from Dir and Chitral were investigated. VA mycorrhizae were of general occurence in all plants examined except members of the Caparidaceae, Comelinaceae, Cyperaceae, Guttiferae, Rutaceae, Violaceae, Zygophyllaceae and 1-10 plants in other families. Percent root length infected by VA mycorrhizal fungi ranged from 50 to 78 in 6% of the plants and 0 to 48 in the others. Three types of species of Endogonaceae (Glomus mosseae, G. macrocapus, Acaulospora laevis) occurred frequently. Spores of G. mosseae were most abundant.

Introduction

A study of the occurence of mycorrhizal associations in plants and Endogon-accous spores in soil of Northern Areas of Pakistan is currently under active investigation. Results for plants and soil samples from Gilgit, Hanza, Nagar and Kaghan valley have been reported (Saif & Iffat, 1976; Saif & Parveen, 1976). The present study is concerned with Dir and Chitral.

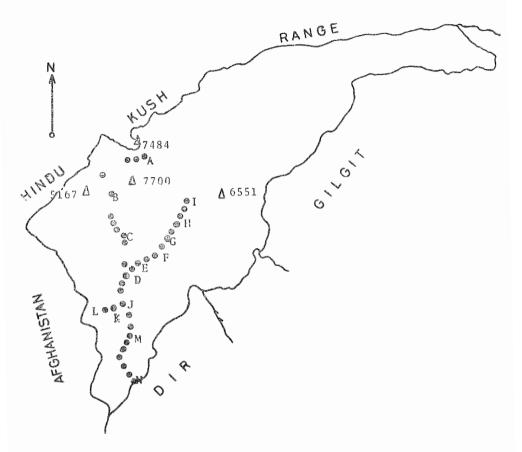
Materials and Methods

Collection of Plants and Soil Samples

Plants aud soil samples were collected from different sites in Dir and Chitral in July and August, 1976 (see Fig. 1 and 2) from cultivated and non-cultivated areas, grasslands, forests and high altitude places e.g.. Lowari Top (3200m). Shahi Top (2438m), Shogore (2204m), Kafiristan Top (2438m). The Chitral district is bounded on the north, south and the west by Afghanistan and is separated from the Soviet Union by a narrow strip of Wakhan, a province of Afghanistan, and from China by Hunza area. It lies between latitude 35° 51N and longitude 71° 47E. The elevation of the area is between 128 to 7700m. It is extremely cold in winter and minimum and maximum temperatures in the main towns of Drosh and Chitral are 11.6°C and 61°C respectively. There is snow fall during winter. During summer i.e. June to August the temperature ranged from 37°C to 53°C.

Three to five plants of each species growing in close proximity were carefully dug up with soil attached to their roots and transported to the laboratory. Plant roots after separation were fixed in formalin/acetic/alcohol (5:5:90).

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Location of plant and soil sample collection in Chitral for VA mycorrhizae and Endogon-accous spores. A, Garam Chashma; B, Arkari; C, Shoghore; D, Chitral; E, Kari; F, Koghozi; G, Maroi; H, Barenis; I, Reshun; J, Ayun; K, Kafiristan Top; L. Bumburet. M, Drosh; N, Lowari Top; Δ. Mountain Peak with altitude in m.

Extraction of Endogonaceous Spores

For extraction of spores 25g of sieved soil was suspended in a 500 ml beaker and stirred well with a glass rod and allowed to stand for 3-5 min. The soil suspension was then poured from the beaker which was simultaneously rotated on to a filter paper placed in the glass funnel. The extraction procedure was repeated again. The spores along with small amount of organic debris thus collected on the filter paper were counted on a stereoscopic microscope as described by Khan (1971).

Measurement of Infection

From pooled samples of the roots, the extent of mycorrhizal infection was measured by recording i) the percentage of root pieces infected and ii) percent root length infected, after clearing fifty root segments for 15-30 min in KOH (10%, at 96°C) and then staining with 0.05% trypan blue in lactophenol (Phillips & Hayman, 1970).

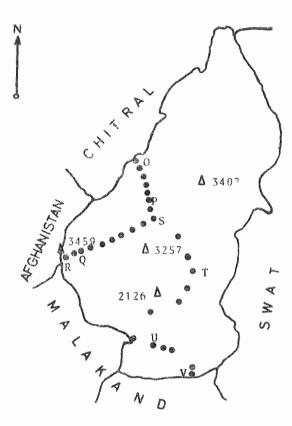


Fig. 2 Location of plant and soil sample collection in Dir for VA mycorrhizae and Endogonaceous spores. G, Gujar Lavv Post; P, Dir; Q, Shahi Top; R, Binshahi; S, Chutiatan; T, Akhgram: U, Sarai: V. Chakdarra Fort; Δ. Mountain Peak with altitude in m.

Result and Discussion

Many plants collected from Dir and Chitral were heavily mycorrhizal, and soil surrounding their roots contained spores of one to four species of Endogonaceae (Table I).

All the soil samples contained spores of endophytic fungi except two associated with Impatiens bicolor (from Garam Chashma) and Polygonum nepalense (from Lowari Top) (Table 1). Glomus mosseae and Glomus macrocarpus var. macro carpus were the most abundant species, therby confirming previous reports (Khan, 1971; Saif & Iffat, 1976; Saif & Perveen, 1976). Out of about 200 species belonging to 47 families, 64 were observed non-mycorrhizal but the soil surrounding their roots contained species of one to four Endogonaceae species (Table 1).

Amaranthaceae:

All the seven species of this family collected from various locations showed mycorrhizal infection which ranged from 1% to 40% whereas spores of Er dogonaceae speies ranged between 8 to 49 per 25g soil. Spores of Acaulospora laeria were asso-

TABLE 1. Mycorrhizal infection in plants and numbers of spores of Endogonaceae in soil samples collected at various locations in Dir and Chitral (July and August, 1976.)

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Dota is mean of 3 replicate Plants. Site of collection is given in Fig 1 & 2. F=flowering; S=Seed.

EXPLANATION OF PLATES

All the figures show portions of mycorrhize I roots from different plants collected from Dir and Chitral. They are not sections but whole segments of roots squased under cover slips after clearing, softening and staining with KOH, trypan blue.

Plate 1.

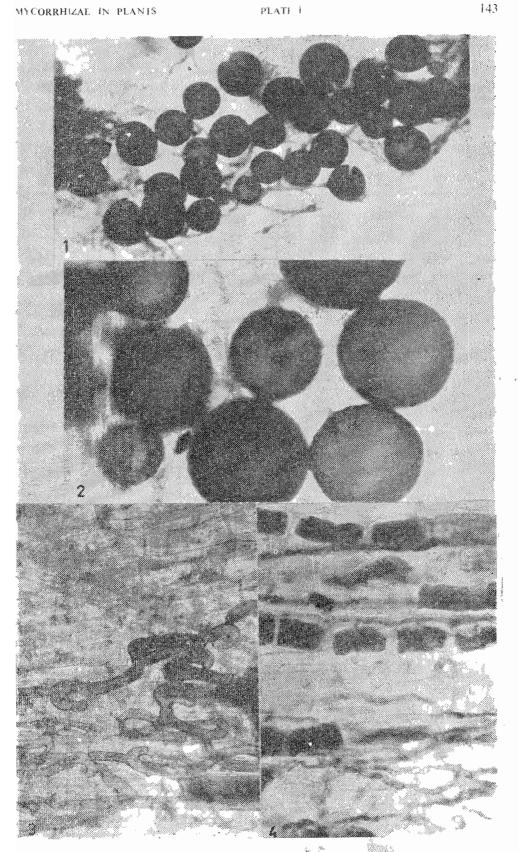
- No. 1. Root cortex with large number of vesicles in Artemesia brevifolia X100.
- No. 2. Portion of root cortex with large number of vesicles in A. brevifolia X 403.
- No. 3. Hyphal coils in the root cortex in Taraxacum officinale X 100.
- No. 4. Cortical cells showing large characteristic arbuscules in *Mentha longifolia* X 100.

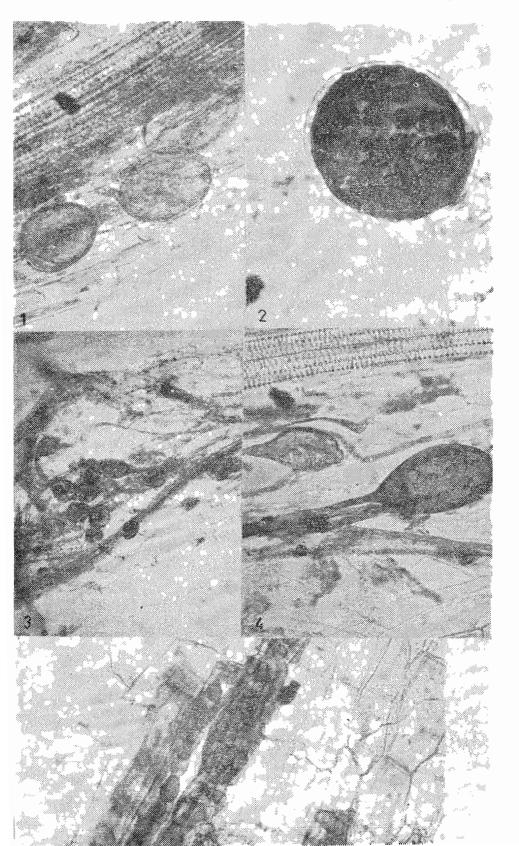
Plate 2.

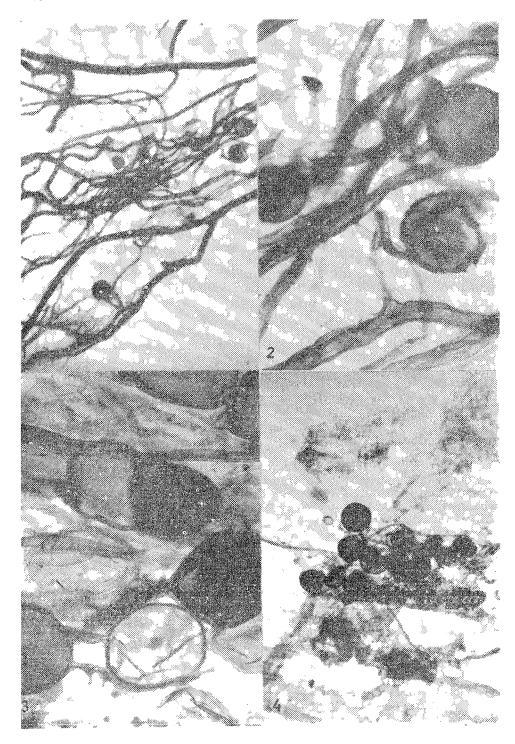
- No. 1. Cortical cells showing vesicles in Plantago lanceolata X 100.
- No. 2. An unidentified structure with appendages on the external surface and small rounded bodies inside associated with roots of *Viola* sp. X 400.
- No. 3. Cortical cells with large number of vesicles in Lactuca seriola X 100.
- No. 4. Vesicles full of oil globules in Ranunculus laetus X 150.
- No. 5. Root cortex showing a septate endophyte of unknown identity forming anastomoses in *Viola* sp. X 400.

Plate 3.

- No. 1. Large amount of external mycelium of a mycorrhizal Fungus in *Myricaria* germanica X 100.
- No. 2. Portion of No. 1 at higher magnification X 450.
- No. 3. Large double walled vesicles in root cortex of *Plantago major* X 400.
- No. 4. Vesicles of a mycorrhizal fungus in *Pulicaria crispa* X 100.







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ciated with only two species (from Garam Chashma) and Digera muricata (from Ayun). Mycorrhizal infection in this family has also been reported for plants collected from other natural habitats, namely Gilgit (Saif & Iffat, 1976) and Kaghan valley (Saif & Parveon, 1976).

Compositate:

Thirty two species were examined for VA mycorrhizal infection. Ten were non-mycorrhizal but the soil around their roots contained different types of Endogonaceae spores (Table I). Spores of G. mosseae were most frequent whereas those of Acaulospora laevis were very rare. Artemisia brevifolia (from Barenis) showed heavy mycorrhizal infection and the root cortex was full of rounded vesicles of a mycorrhizal fungus (Plate 1. No. 1-2) Taraxacum officinale (from Koghozi) was also heavily infected (59% root length infected). Fungal coils were observed in the root cortex (Plate 1, No. 3). Lactuca serriola (from Shoghore) was also found heavily infected with 58% root length infected and also contained in the root cortex vesicles of mycorrhizal fungus (Plate 2, No. 3). Plate 3, No. 4 shows vesicles of mycorrhizal fungus in Pulicaria crispa (from Chitral) whereas value for percent root length infected was 54 (Table 1). Characteristic of VA mycorrhizal infection in other host species were similar to those reported in earlier studies (Saif & Iffat, 1976: Saif & Parveen 1976).

Labiatae:

Out of eight species of Labiatae collected from various locations in Dir and Chitral, Nepeta podostachys (from Gujar Lavy) & Slachys serices lacked mycorrhizal infection. In other species mycorrhizal infection ranged between 1 to 78% (Table 1) Mentha longifolia, ssp. longifolia (from Chitral) showed typical arbuscular infection (Plate 1, No. 4). Soil around the root of these plants showed presence of three to four types of spores of Endogonaceae.

Plantaginaceae:.

Out of eight species, only *Plantago major* (from Lwari Top) lacked mycorrhiza but the soil around its roots contained 70 spores/25 gm soil. Roots of *P. major* (from Chitral) contained many vesicles. Vesicles showed funnel-shaped bases whereas fungal hyphae were smooth surfaced (Plate 3. No. 3). *P. lanceolata* also showed vesicular infection (Plate 2. No. 1).

Polygalaceae;

A single species of Polygalaceae i.e. Polygala siberiea was collected from Dir and its roots were found infected with mycorrhizal fungus (6% root length infected) and the soil around its roots contained three types of spores (Table 1). Similar findings have been reported by Saif (1975) who described P. arvensis harbouring mycorrhizal infection under natural conditions.

Polygonaceae:

Eleven species of Polygonaceae were collected from different locations in Dir and Chitral and only two, *Polygonum aviculare* (from Bumburet). *P. paronychioides* (from Lowari Top) were non-mycorrhizal. Result for other species were similar to previous observations (Saif & Iffat, 1976; Saif & Perveen, 1976).

Ranunculaceae:

Six species were examined and one of them, namely Ranunculus laetus (from. Drosh and Chitral) were non-mycorrhizal. Clematis grata (from Shahi Top) showed 36% root length infected (Table 1) and R. laetus (from Garam Chashma) although not heavily infected showed elliptical vesicles full of oil droplets and smooth surfaced fungal hyphae (Plate 2, No. 4).

Rosaceae

Six species of Rosaceae were collected of which two, Filipendula vestita (from Bumburet) and Sorbaria tomentosa (from Kafiristan Top) were non-mycorrhizal although the soil around their roots had 65 and 25, spores/25 gm soil (Table 1). Geum urbanum (from Shahi Top) and Poterium polygonum (from Dir) showed 1% root length infected whereas Rubus sp. (from Shahi Top) had 19% root length infected (Table 1).

Tamaricaceae

A single species of Tamaricaceae i.e. Myricaria germanica (from Koghoz.) showed 84% root segments infected and 44% root length infected. Root of this plant showed a large amount of external mycelium with spores borne on short branches (Plate 3, No. 1-2). Fungal hyphae were broad, thickwalled and rough surfaced.

Umbelliferae

Thirteen species of Umbelliferae were examined for mycorrhizal infection and, of those, Bupleurum tenue (from Shahi Top), Dacus carota (from Kafiristan Top) Ergagium sp (from Dir), Pimpinella anisum (from Shahi Top), Scaligeria chitrallia (from Kafiristan Top) and Seseli lebantis (from Lowari Top) were non-mycorrhizal but the soil around their roots contained spores of Endogonaceae (Table 1) Host species showed percent root length infected in the range of 3 to 72 (Table 1). Characters of endophytic fungi in other species were similar to those reported by Saif & Iffat (1976) and Saif & Parveen (1976).

Violaceae

Roots of Viola species (from Shahi Top) were infected with a fungus with septate hyphae, and forming anastomoses in the cortical cells (Plate 2, No. 4). The nature of this fungus i.e. whether it is mycorrhizal or not is not known. Another structure with large number of small rounded bodies inside was observed with the roots of this plant (Plate 2, No. 2). The structure also have appendages on the external surface. The nature of this structure could not be ascertained.

Members of families e.g. Caparidaceae (Caparis spinosa (from Reshun), Cleome viscosa (Drosh). Comelinaceae (Comelina banghalensis (Dir), Cyperaceae (Cyperus rotundus (Ayun), Guttiferae (Hypericum perforatum (Lowari Top)), Rutaceae (Haplophyllum Superpositum (Ayun)), Violaceae (Viola sp.) (Shahi Tof) and Zygophyllaceae (Tribulus terrestris (Arkari & Chakdarrah)) were found to be non-mycorrhizal, although members of some of these fami ies have been reported as mycorrhizal previously (Saif, 1975; Saif & Perveen, 1976), e.g., Comelina banghalensis (Comelinaceae), Cleome viscosa (Caparidaceae) Cyperus niveus (Cyperaceae) (Saif, 1975). Hypericum perforatum a member of Guttiferae collected from Shogran (Kaghan Valley) was mycorrhizal (Saif & Perveen, 1976). This as well as previous studies

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(Saif, 1975; Saif & Naheed, 1976; Saif & Parveen 1976) indicates that host species even of same family may vary in mycorrhizal infection depending upon different localities and habitats. If one species is mycorrhizal at one place it may be non-mycorrhizal at other place. This is perhaps due to the fungus flora available for infection in different habitats, its quantity, and ability to infect perticular types of host species.

The prosent study and previous reports (Saif & Iffat, 1976. Saif & Parveen, 1976) show a wide spread presence of mycorrhizal species of Endogon teac throughout the northern areas of Pakistan. These studies give us an idea on the type, intensity and distribution of indigenous types of mycorrhizal species of Endogonaceae present in the northern areas of Pakistan.

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