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

1-Hunza, Nagar and Gilgit

By

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Summary

The incidence of vesicular-arbuscular mycorrhizal in the roots and Endogonaceous spores in rhizosphere of 128 plants belonging to 37 families collected from Hunza, Nagar and Gilgit States of Pakistan was investigated. Vesicular-arbuscular mycorrhizal were of general occurrence in all types of plants except few. VA mycorrhizal infection was also observed in 30 members of 10 families which were generally considered as non-mycorrhizal e.g. Amaranthaceae (*Amaranthus hybridus*), Chenopodiaceae (*Chenopodium album, C. botrys, C. foliosum, Kochia indica*), Caryophyllaceae. (*Cerastium fontanum, Stellaria media, Silene vulgaris, Arenaria stercularia*), Cruciferae (*Capsella bursapastoris, Rorippa indica*), Euphorbiaceae, (*E. falcata, E. prostrata*), Oxalidaceae (*Oxalis corniculata*), Polygonaceae (*Polygonum aviculare, P. gilssii, P. nepalense, R. hastatus, Fagopyrum tataricum*), Rahnunculaceae (*Clematis orientalis, Rannunculus* sp.) and Urticaceae (*Urtica ardens*). In most of the plants arbuscular infection was very poor in contrast to large number of vesicles present in the root cortex. Four different types of mycorrhizal endophytes, three being Endogonaceous and one with brown, septate, narrow hyphae were observed. Six types of Endognonaceous spores were observed in the soil samples. Rhizosphere soil of most of the plants contained large number of 2-4 types of spores with *Glonus mosslae* and *Glomus macrocarpus* in great abundance reaching up to 2579/50 gm rhizosphere soil (*Salvia nubicola*, a member of Labiatae).

Introduction

Vesicular-arbuscular (VA) mycorrhizae occur on most cultivated crops and many herbaceous and woody non-cultivated plants of Pakistan. Khan (1971) made first study for occurrence of spores of Endogonaceae in West Pakistan soils covering mostly the plains of Punjab and 2-4 locations from the other provinces. Eight types of spores were reported in his study. Najma et al., (1971) made a survey of 13 angiospermous trees growing around New University Campus, Lahore, and found these plants with mycorrhizal infection. Khan (1974) further, surveyed incidence of mycorrhizal infection in the roots and spores in rhizosphere soil of 52 xerophytes, 21 halophytes and 16 hydrophytes collected from plains, the salt ranges, the coastal regions and the deserts of Punjab, Sind and Baluchistan provinces of Pakistan. Saif (1975) made a survey for occurrence of mycorrhizas in 75 plant species growing around University Campus, Islamabad and Endogonaceous spores in their rhizosphere soil.

However, a vast area of the country has not yet been surveyed for vesicular-arbuscular (VA) mycorrhizal associations and Endogonaceous spores. Due to the importance of the symbiotic associations that participate in the fixation and uptake of phosphorus in pioneer colonization of nutrient deficient regions (Harley, 1970) it has been planned to make a thorough survey for the occurrence of these endophytes in different regions of Pakistan. Present survey included Gilgit (Eastern Part only), Hunza and Nagar which have season, vegetation and topography of their own and had never been surveyed before for mycorrhizal associations in plants and Endogonaceous spores in soils.

Materials and Methods

Collection of plant and soil samples: Plants and soil samples were collected from sites in Gilgit, Hunza and Nagar States in summer from July 5, 1975 to July 23, 1975, covering a wide range of soil types e.g. sides of the rivers (Indus, Gilgit, Hunza), loamy soils, gravely soils, rocks, grasslands and forests of Naltar (alt. 10000 ft). The locations of samples collection are indicated in Fig. 1. Plant and soil samples examined came from cultivated and non-cultivated areas under natural conditions. Three to five plants of each species growing in close proximity were carefully dug up with soil attached to their roots and placed separately in polythene bags for transportation. Plant roots were separated from the soil samples, washed, carefully in water to remove the adhering soil and whole root systems were fixed in formalin/acetic/alcohol (5:5:90).

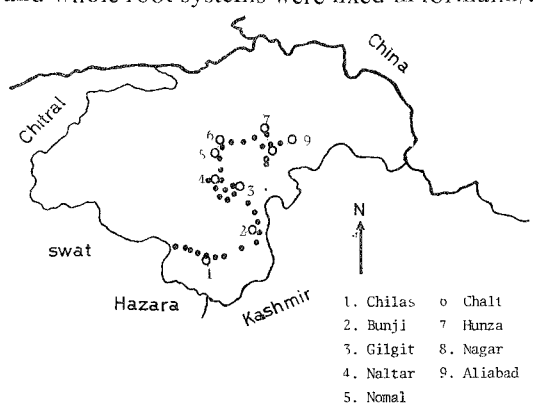

Fig 1 Location of soil samples and plants collected in Gilgit Agency examined for vesicular-arbuscular mycorrhizae and Endogonaceous spores.

Measurement of extent of infection: Root systems of each plant were cut into 1 cm segments, and from these segments, extent of mycorrhizal infection was measured by recording the percentage of root pieces infected after clearing fifty 1cm root segments for 30 min in KOH (10% at 90°C) and then staining with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970).

Extraction of Endogonaceous spores: Endogonaceous spores were recovered from the sieved soil samples by taking 3 sub-samples each weighing 50g from each soil sample. Each sub-sample was placed in 200 ml of water contained in 500 ml beaker, stirred well with magnetic stirrer for about 2-4 min and allowed to stained for about 3-5 min to permit large soil particules to settle at the bottom. The supernatent was then poured from the beaker which was simultaneously rotated onto 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 stereiscopic microscope as described by Khan (1971).

Identity of Endophytes: Different types of endophytes present inside the root cortex as well as external mycelium on the root surface were identified by the

morphological characters of external as well as internal mycelium, staining reaction, shape and form vesicles, any other specific structure produced by the mycelium and also by comparing them with descriptions given by different workers (Daft and Nicolson, 1974; Furlan and Fortin, 1973; Butler, 1939; Mosse and Hayman, 1971).

Results

Gilgit is situated in the north east corner of Pakistan and consists of Gilgit, Chilas, Hunza, Nagar, Punnial, Political Districts of Iskoman, Kohi-i-Ghizar, Yasin, and Darel. The climate of the area is arid and the weather is extreme, especially the winter is severely cold. During the summer the temperature sometimes goes upto 106°F. Rainfall ranges from 3-5″ annually. The mountains are barren and dry except some vegetation in the middle of the ridges. The valley itself is well cultivated and vegetated. The general elevation of the valley is 4000 ft to 5000 ft. The area surveyed included Kara Karum range.

Type and numbers of Endogonaceous spores: The results of this survey are presented in Table I. Most of the soil samples contained Endogonaceous spores as regular components of their microflora. Soil samples collected showed presence of six different types of spores. They belonged to the family Endogenaceae, size of each spore type given in parenthesis with the name, *Glomus mosseae* (150-260 μm), *G. macrocarpus* (140-210 μm) *G. fasciculata* (70-110 μm), *Gigaspora calospora* (90-210 μm), *Acaulospora laevis* (110-230μm) and funnel-shaped spores (145-265 μm) (Gerdemanin and Trappe, 1974). Three to four types of spores were observed in most of the soil sampels and spores of *G. mosseae* were in greater number as compared to the other types.

Rhizosphere samples from *Cynanchum acutum*, *Cynoglossum lanceolatum*, *Chenopodium album* (collected from Hunza), *C. foliosum* (from Hunza), *Kochia indica* (from Jutal and Hunza), *Artimisia maritima* (from Basain and Nagar), *Cichorium intaybus* (from Hunza), *Convolvulus arvensis* (from Jutal and Basain), *Mathiola flavida* (from Nagar), *Sophora* sp. (from Jutal), *Epipactis helleborine* and *Plantago major* (from Chenar Bagh), *Polygonum amphibium* (from Naltar), *Portulaca oleraceae* (from Jutal), *Clematis orientalis* (from Nagar), *Veronica anagalia aquatica* (from Basain) contained three to four types of Endogonaceous spores although no mycorrhizal infection was observed (Table I).

Members of Chenopodiaceae (9 species) collected from different locations showed Endognoaceous spore number ranging from 15 to 527/50 gm soil. *Glomus fasciculata* was abesent from the rhizosphere samples of Chenopodiaceae. Members of Cruciferae showed 0-135 Endogonaceous spores/ 50 gm soil, member of Polygonaceae 0-431, Rannunculaceae, 37-72, Euophorbiaceae 114-287 spores/50 gm soil. *Salvia nubicola*, a number of Labiatae showed highest number (2579/50 gm soil) of Endogonaceous spores in their rhizosphere soils.

Vesicular-arbuscular mycorrhizal infection: Vesicular-arbuscular (VA) mycorrhizas were of general occurrence in all the families except in a single family e.g. Asclepiadaceae although rhizosphere soil of one of its member (*Cynanchum acutum*) contained Endogonaceous spores (Table I). This however, does not indicate that this family is non-mycorrhizal because presence of mycorrhizal infection has been reported (Khan, 1974). In the same family different genera and species differed with each other for the intensity of infection and percentage root segments mycorrhizal (Table I).

Table 1. Mycorrhizal development in plants and number of spores of Endogonaceae in soil samples collected at various locations in Gilgit, Hunza and Nagar between July 5 to July 23, 1975.

Family genus, species	Growth stage.	Site of collection	% root segments mycorrhizal	Intensity of infection.	Spore number/50 g				Total
					Glomus mosseae	*Glomus marcocapus.*	*Glonmus fasiculatus.*	Other types of spores	
Amaranthaceae									
Amaranthus hybridus L.	F	C.B	98	+++	173	113	39	3	328
Asclepiadaceae									
Cynanchum acutum L.	F	N	—	—	130	38	—	—	168
Balsaminaceae									
Impatiens brachycentra Kar & Kir.	F	N	98	+++	56	23	—	—	79
Boraginaceae									
Cynoglossum lanceolatum Forssk	F	C.B	—	—	53	32	—	2	87
C. lancolatum Forssk.	F	Na	100	+++	9	1	—	—	10
Heliotropium europaeum var. lasiocarpum (Fish. & Mey.) Kazmi,	F	G	35	+	2	8	—	—	10
H. crispum Desf. lasicrpum (Fish.& Mey.)	F	Na	60	+	20	226	22	22	270
Paracaryum moltikiodies Roy. in Banth.) Kazmi.	F	Na	85	+	15	10	—	5	30
Caryophyllaceae.									
Cerastium fontanum Baumg.	G	J	35	+	300	112	45	—	457
Stellaria media (L.) Cyr.	F+S	B	25	+	20	16	—	—	36
Silene vulgaris (Moench) Garcke	F	Na	55	+	6	4	—	—	10
Arenaria serpyllifolia L.	F	Na	50	+	270	25	49	—	344
Cannabinaceae									
Cannabis sativa L.	V	N	90	—	53	5	—	—	58
Chenopodiacae									
Chenopodium album L.	+S	C.B	100	+	26	214	—	—	240
C. album L.	V	B	65	+	72	87	—	—	159
C. album L.	V	H	—	+	35	50	—	—	85
C. botrys L.	V	TD.	50	+	210	44	—	7	261
C. botrys L.	F	C.B	95	+++	6	4	—	—	10
Kochia indica Wight.	V	N	5	+	10	—	—	—	10
K. indica Wight.	V	J	—	—	243	284	—	—	527
K. indica Wight.	V	H	—	—	15	2	—	—	7
Chenopodium foliosum (Maench) Aschers.	V	H	—	—	7	5	—	3	15

Compositae										
Artemisia dracunculus L.	V	G	95	+	122	23	—	3	148	
A. maritima L.	F	B	—	—	6	—	—	—	6	
A. maritima L.	F	N	—	—	42	10	—	—	52	
A. rox-burghiana Wall. et Basson.	F	H	96	+++	234	84	50	—	368	
A. sp.	V	N	55	+	46	22	—	—	68	
Cirsuium arvense L.	F	T.D.	95	+	91	3	—	—	94	
C. arvense L.	F	N	65	+	11	28	—	—	39	
Cichorirum intybus Linn.	F	H	—	—	—	35	—	—	35	
Conyza bonariensis Cronquist	F	C.B	90	+++	15	17	—	—	32	
C. canadenses (L.) Cronguist	F	Na	93	++	21	19	—	5	45	
Myriactis wallichii Less.	F	C.B	94	+	15	3	—	—	18	
M. wallichii Less.	F	C.B	80	+	68	205	—	1	274	
Lactuca serriola L.	F	C.B	10	+	80	65	49	—	164	
Leontopodium sp.	F	Na	20	+	17	22	—	7	46	
Taraxacum officinale Wogg.	V	G	93	+	270	80	—	—	350	
T. officinale Wogg.	F	N	92	++++	120	105	60	0	285	
Tussilago farfara L.	V	N	99	++	2	5	—	—	7	
Sonchus arvensis var. brachyotus (DC) Kirp.	F	B	98	++++	25	17	—	—	42	
S. arvensis. L.	F	N	90	+++	70	45	—	—	115	
S. arvensis. L.	F	N	98	++++	183	50	—	—	233	
S. oleraceus L.	F	B	50	+	15	17	—	—	32	
Saussurea albescens (DC) Kirp	F	Na	50	+	50	4	—	—	54	
Convolvulaceae										
Convolvulus arvensis L.	F	J	—	—	90	21	—	—	111	
C. arvensis L.	F	N	10	+	20	26	15	—	61	
C. arvensis L.	F	B	—	—	—	2	—	—	2	
Cruciferae										
Capsella bursapastoris L.	F+S	N	98	++	72	63	—	—	135	
Mathiola flavida Boiss.	F	N	—	—	56	24	20	—	100	
Nasturtium officinale R. Br.	F	C.B	—	—	—	—	—	—	0	
Rorippa indica (L.) Hiern.	F+S	C.B	70	+	12	15	—	—	27	
Elaegnaceae										
Hippophae rhamnoidese L.	S	N	85	+	83	31	30	—	144	
H. rhamnoidese L.	V	G	96	+++	241	205	—	—	446	
Equisetaceae										
Equisetum palustre L.	V	H	99	++	199	80	—	—	279	
E. palustre L.	V	Na	90	+	112	45	11	2	170	
Euphorbiaceae										
Euphorbia falcata L.	S	J	93	++++	100	160	27	—	287	
E. falcata L.	S	T.D	96	++	220	500	30	—	750	
E. prostrata Ait.	S	J	80	+	58	56	—	—	114	
Filicales										
Adiantum capillus-veneris L.	V	C.B	98	++	35	45	—	—	80	
A. capillus-veneris L.	V	C.B	75	+	45	13	—	—	58	
Geraniaceae										
Centourium meyeri (Bunge) Druce.	F	N	92	++	4	—	—	—	4	

Hippuridaceae									
Hippuris vulgaris L.	S	N	94	+	141	40	—	—	181
Gramineae									
Triticum vulgare L.	F	N	95	++	54	14	—	—	68
Paplionaceae									
Alhagi camelorum Fisch.	V	J	—	—	130	147	23	—	300
Astragalus graveolens Buch-Ham.	F	Na	95	+	20	22	—	—	44
A. pilocentros Fisch.	V	G	—	—	16	6	—	—	22
A. scorpiurus Bunge.	F	Na	65	+	25	14	—	—	39
A. sp.	V	N	99	+++	452	354	—	—	806
Sophora sp.	V	J	—	—	320	217	50	—	587
S. mollis (Royle) Baker	V	G	65	+	140	40	22	—	202
Lotus corniculatus L.	F	G	85	+	24	20	—	—	44
Labiatae									
Prunella vulgaris L.	F+S	C.B	98	+	3	1	—	—	4
Mentha longifolia L. Huds.	F+S	H	97	++	305	95	—	—	400
M. longifolia L. Huds.	V	B	95	+	32	25	—	—	57
Salvia nubicola Wall. ex Sweet	F	Na	90	+	2132	142	300	5	2579
Oxalidaceae									
Oxalis corniculata L.	S	G	93	+	100	36	25	—	161
Onagraceae									
Oenothera biennis L.	F	G	94	+++	16	9	—	—	25
Orobanchaceae									
Orobanche cernua var. hanssi G. Bech	F	N	93	+	170	30	—	—	200
Orchidaceae									
Epipectis royleana Lindl.	F	Na	98	+	51	151	—	—	202
E. helleborine (L) Crantz.	F	C.B	—	—	—	2	—	—	2
Spiranthes lencea (Thunb.) Baker	F	C.B	99	+	1	2	—	—	3
Zeuxine strateumatica (L.) Schlechter.	V	Na	45	+	13	3	—	—	16
Paranassiaceae									
Parnassia palustris L.	F	N	99	+	60	12	—	—	72
Plantaginaceae									
Plantago lanceolata L.	F+S	C.B	10	+	70	37	24	—	131
P. major L.	F	N	89	+++	—	4	—	—	4
P. major L.	F	C.B	—	—	10	15	13	2	40
P. major L.	F	C.B	93	+	27	22	—	—	49
P. major L.	F	Na	92	++	36	3	—	7	46
Polygonaceae									
Polygonum amphbium L.	F	Na	—	—	82	26	—	—	108
P.aviculare L.	F	H	97	+++	30	19	—	—	49
P. aviculare L.	F	N	98	+	176	35	—	—	211
P. convolvulus L.	F+S	T.D	99	+	91	3	—	—	94
P. gilessi Hemsl.	F	Na	45	+	174	32	—	3	209
P. nepalense Meissn.	F	N	89	+	—	—	—	—	—
P. nepalense Meissn.	F	H	65	+	200	201	30	—	431
P. nepalense Meissn.	V	C.B	85	+	11	4	5	—	20
Rumex hastatus D. Dong	F	G	85	+	4	16	—	—	20
R. crispus L.	F	Na	97	+					
Fagopyrum tataricum (L) Gaertn.	S	T.D	55	+	380	30	50	—	460

Portulacaceae									
Portulaca oleraeae L.	S	J	—	—	—	4	—	—	4
P. oleracea L.	V	B	20	+	7	115	—	—	122
Rubiaceae									
Rubia cordifolia L	F+S	Na	99	+	68	46	—	—	114
Rosaceae									
Potentilla sp.	V	N	68	+	45	11	—	—	56
P. sp.	F+S	N	85	+	18	7	—	—	25
P. reptans L.	F	Na	30	+	60	556	22	—	138
Ranunculaceae									
Clematis orientalis L.	F	N	—	—	21	16	—	—	37
Ranunculus sp.	F+S	N	99	+	62	10	—	—	72
R. sp.	F	Na	99	+	25	16	8	—	49
Solanaceae									
Datura stramonium L.	F	F	75	+	30	9	—	—	39
Hyoscaymus nigar L.	F+S	N	95	+	48	33	—	—	81
Nicotiana sp.	F	G	97	+	2	—	8	—	10
Solanum nigrum L.	F	C.B.	98	+	6	—	—	—	6
Scrophulariaceae									
Veronica anagallis aquatica L.	V	B	—	—	16	13	4	—	33
V. anagallis L.	F	N	20	+	31	15	13	—	59
Verbascum thapsus L.	F+S		94	+	11	3	—	—	14
Sterculiaceae									
Swertia cordata (G. Don) Clarke	F	H	85	+	268	92	—	—	360
Thymeleaceae									
Thymelaea passerina L. Coss & Germ.	F	C.B.	98	+++	5	2	—	—	7
T. passerina (L.) Coss & Germ.	F	Na	65	+	25	250	—	—	275
Umbelliferae									
Heracleum candicans Wall ex.DC	F	Na	50	+	60	342	—	—	402
Pimpinella diversifolia (Wall) D.C	F	Na	98	+++	34	34	—	—	68
Vicatia wolffiana (wolff & Fedde.)	F	Na	55	+	49	46	14	—	109
Bupleurum falcatum L.	F+S	Na	55	+	50	30	—	—	80
Urticaceae									
Urtica ardens Link	F	Na	88	+	27	34	—	—	61
Urtica sp.	V	B	97	+	68	46	—	—	114

* = Mean of three replicates.

** =V; Vegetative; F. flowering, S; Seed.

*** = +, Low; + +, medium; +++; high; + + + very high

**** =C. B; Chenar Bagh. N: Nagar, Na; Naltar, G; Gilgit, J; Jutal, B; Bassain, H; Hunza. T.D: Tung Das.

Among the 9 species of Chenopodiaceae, four were observed as non-mycorrhizal whereas four showed mild infection and one *Chenopodium botrys* showed high level of infection. Plate 4, No. 3 shows presence of VA infection in *C. botrys*.

Twenty two plants belonging to 11 genera of Compositae were sampled for study of VA mycorrhizal infection (Table 1). Out of these only 3 species namely, 2 species of *Artimisia* (from Basain and Nagar) and *Cichorium intybus* (from Hunza) were non-mycorrhizal, 12 plants showed very little infection, 3 plants high level of infection. Four plants namely (*Taraxacum officinale*) (from Nagar), *Sonchus arvensis* (from Basain), *Sonchus arvensis* (2 plants, from Nagar) showed very high VA mycorrhizal infection (Table 1). In *T. officinale* cortex cells were densely filled with arbusscules and elliptical to oval vesicles were observed in the inner cortex. Rhizosphere of this plant contained large amount of groups of Endogonaceous spores (Plate 5, No. 3). Spores with septate subtending hyphae were observed on the root surface (Plate 5, No. 4). Vesicle were also observed in *S. arvensis* (Plate 4, No. 2). *Myriactis wallichii* (from Chenar Bagh) showed 80-94% root segments infected whereas intensity of infection was low. Root cortex showed vesicle formation and another type of vesicle with reticulate structures was also present (Plate 5, No. 1-2).

Among the four members of Cruciferae namely, *Capsella bursapastoris* (from Nagar) and *Rorippa indica* (from Chenar Bagh) showed VA mycorrhizal infection whereas *Mathiola flavida* (from Nagar) and *Nasturium officinale* (from Chenar Bagh) showed no mycorrhizal infection (Table 1).

Among the 3 members of Euophorbiaceae, *Euphorbia falcata* (from Jutal) showed 93% root segments infected with very high intensity of infection (Table 1). Root cortex was filled with large number of vesicles (Plate 2, No. 1) of mycorrhizal fungus. Vesicles were mostly rounded in shape and were borne terminally at the end of small hyphal branches given off by the main hyphae (Plate 2, No. 2). Fungal hyphae in the root cortex were smooth surfaced and were without any angular projections (Plate 2, No. 2). *E. falcata* (from Tung Das) and *E. prostrata* (from Jutal) showed 96% and 80% root segments infected respectively and their roots were mildly infected with mycorrhizal fungus.

Members of Rannunculaceae showed chracteristic VA mycorrhizal infection (Plate 2, No. 3-4). *Clematis orientalis* (from Nagar) showed no mycorrhizal infection whereas *Rannunculs* sp. (from Nagar and Naltar) showed very high percentage of root segments infected (99%) with very low intensity of infection (Table 1). Large number of vesicles with different shapes were found filling the root cortex (Plate 2, No. 3-4). In some cases the old vesicles appeared shrinked and deformed (Plate 2, No. 3) whereas in others they appeared full of cytoplasmic contents (Plate 2, No. 4). The nature of these cytoplasmic contents is not clear whether these vesicles contained oil droplets or vacuoles, however, rounded structures are visible (Plate 2, No. 4).

Among the 12 members of Polygonaceae (a family generally considered non-mycorrhizal, Gerdemann, 1975) only one plant namely, *Polygonum amphibium* (from Nalter) was non-mycorrhizal, *Polygonum aviculare* (from Hunza) showed 97% root segments infected and high intensity of infection whereas rest of the members showed less intensity of infection with 45-98% root segments infected. Plate 4, No. 1 showed portion of the root of *Rumax hastatus* with large amount of external mycelium and few Endogonaceous spores. Root cortex was observed with many vesicles. *Fagopyrum tataricum* (from Tung Das) showed 55% root segments infected whereas intensity of

infection was low but few pieces were very heavily infected with VA mycorrhizal fungus (Plate 1, No. 1-3). Before penetration fungal hyphae form appressoria on the surface of root from which hyphae are distributed towards different direction in the root cortex (Plate 1, No. 1). In some cases root cortex contained two types of vesicles, one type of typical vesicle which are characteristic of VA mycorrhizal fungus and another reticulated type of vesicle. The nature of the fungus producing such types of vesicles is not known. It may be entirely a new genus or species of some genus belonging to Endogonaceae not yet known (Plate 1, No. 2). Large amount of internal mycelium and vesicles were also observed in the root cortex (Plate 1, No. 3).

Among the members of Solanaceae, in case of *Nicotiana* sp. germinating Endogenaceous spores were observed on the root surface (Plate 3, No. 3). Also, many vesicles of different shapes and sizes were observed in the root cortex (Plate 3, No. 4). Vesicles observed were of irregular shapes. Two plants belonging to Urticaceae, a family reported to be non-mycorrhizal (Gerdemann, 1975) were found infected with mycorrhizal fungus. *Urtica ardens* (from Naltar) and *Uritica* sp. (from Basain) showed 88 and 97% root segments infected respectively whereas intensity of infection was low (Table 1). Very few vesicles were observed in the root cortex. *Plantago major* (from Nagar) a member of Plantaginaceae showed high mycorrhizal infection and the root cortex were observed with large number of vesicles and internal mycelium (Plate 1, No. 4-5). In some pieces only mycelium was observed and no arbuscule or vesicle (Plate 1, No. 5). The internal mycelium in such cases showed no angular projections but in contrast was smooth surfaced (Plate 1, No. 5). *Hippuris vulgaris* (from Nagar), a member of Hippuridaceae showed root cortex full of oblong vesicles of different sizes and no arbuscules were observed (Plate 4, No. 4).

Members belonging to the other families listed in Table I showed various degrees of VA mycorrhizal infection with various percentages of root segments infected. They all showed more or less same characteristics of VA mycorrhizal infection as reported for other families.

Four different types of endophytes were observed in the plant roots. Most commonly occurring endophyte was identified as *Glomus mosseae*. The hyphae of this fungus were darkly stained with trypan blue, had characteristic angular projections, large arbuscules and rounded vesicles. Two types were identified as *Glomus marcrocarpus* and *Glomus fasiculata*. Another type was observed in most root systems with narrow, septate brown hyphae and not stained by trypan blue as compared to Endogonaceous endophytes. This endophyte sometimes after repeated division at one terminal and produced reticulate structures in the cortical cells and sometime start budding so that beaded structures appear in the cells.

Discussion

The present survey showed that Endogonaceous fungi and spores are found abundantly in plants and soil samples collected from Hunza, Nagar, and Gilgit. It is also evident from this study that the degree and type of mycorrhizal infection varies from place to place and the growth stage among the same genus as well as same species.

This survey indicates that Endogonaceous spores are widely distributed in the areas of Hunza, Nagar and Gilgit. Presence of 4-6 types of spores further indicates

that these types have very wide range of occurrence. Among different types of spores observed spores of *Glomus mossae* were in much large number and the observations are consistant with those of Khan (1971). Spores of *Glomus macrocarpus* were less in number as compared to that of *Glomeus mosseae* but were also very widely distributed. Other spore types e.g. *Gigaspora calospora*, *Glomus fasiculata*, *Acaulospora leavis* (Gerdemann and Trappe, 1974) observed in the soil samples were less in number as compared to other two types. These observations are consistant with those of Khan (1971). It was found that those soils samples that were clayey in nature and collected from cultivated soil contained greater number and types of spores. Khan (1971) also reported occurrence of five spore types in clayey soils of the Indus Plains. The abundance of spore of *G. mosseae* in the clayey soils has also been reported by Mosse and Bowen (1968).

Although plants belonging to families e.g. Euphorbiacee, Chenopodiaceae, Polygonaceae, Urticaceae, Cruciferae, Amaranthaceae have been reported to be non-mycorrhizal (Gerdemann, 1968; Khan, 1972, 1974). In the present study large number of Endogonaceous spores were observed from their rhizosphere soils. Similar results were also observed by Saif (1975) who reported presence of large number of spores in the rhizosphere soils of Euphorbiacee and Polygonaceae, although few spores were observed in some of the other families mentioned above. Majority of the plants were sampled at flowering or seed bearing stage and their rhizosphere contained large number of spores as compared to those that were in vegetative stage. Such observation have also been reported by earlier workers (Hayman, 1970; Saif and Khan, 1975; Saif, 1975b).

During the present survey plants belonging to 37 families were found harbouring mycorrhizal infection. Among these families mycorrhizal infection was observed in Crucifrae, Chenopodiaceae, Amaranthaceae, Euphorbiaceae and Polygonaceae, Urticaceae, although in addition to these, Cyperaceae, and Comelinaceae have also been reported as non-mycorrhizal by Khan (1972, 1974). However, exception have been noted (Gerdemann, 1968) and more recently Saif (1975a) reported mycorrhizal infection in plants belonging to Euphorbiaceae, Comelinaceae, Polygonaceae and Cyperaceae. In addition to this Williams *et. al.*, (1974) Ross and Harper (1973) and Kruckelamann (1973) observed VA mycorrhizal infection in Chenopodiaceae. Mejstrik (1972) reported VA mycorrhizal infection in several species of Cyperaceae. Ross and Harper (1973) and Kruckelmann (1973) also found VA mycorrhizal infection in members of Cruciferae.

Information referring to mycorrhization of Labiatae are consistant with those of Boullard *et al.*, (1962) and Saif (1975a) but in the present studies rhizosphere soil contained much larger number of Endogonaceous spores e.g. 2579/50 gm soil in case of *Salvia nubicola*. Results about mycorrhization of Compositae, Rosaceae, Euphorbiacae, Polygonaceae, Papilionaceae, Rannunculaceae are similar to those reported by earlier workers (Boullard *et al.*, 1972; Saif, 1975a; Khan, 1974; Strzemska, 1975). Presence of VA mycorrhizal infection in Chenopodiaceae, Cruciferae, Urticaceae in the present study and reports by the earlier workers (Williams, *et al.*, 1974; Ross and Harper, 1973 and Daft and Nicolson, 1974) indicate that these families are no more non-mycorrhizal and member of these families can harbour VA mycorrhizal infection under the natural conditions. As more plant species are examined, generalizations about the occurrence of VA mycorrhizae will become more difficult.

Two to three different endophytes were observed abundantly infecting the root systems of plants collected. Plants which were collected from sandy soils usually showed a single dominant species (*Glomus fasiculata*) whereas those from other regions with loamy and clayey soils contained two other species, namely *Glomeus mosseae* and *Glomus macrocarpus*. In addition to the above mentioned endophytes another endophyte of unknown affinity with narrow, brown and frequently septate hyphae was commonly observed in the roots. This endophyte remained unstained with trypan blue and formed frequent anastomoses in the cells. There are reports on the occurrence of such an endophyte (Ali, 1969, Greenall, 1963; Daft and Nicolson, 1974: Mosse and Hayman, 1971; Saif, 1975b) however, in the present study vesicle or arbuscule formation by this endophyte was not observed although it was present frequently intermingled with the other endophytes, occupying the same or adjoining cells. The presence of frequent septa indicates its non-phycomycetous nature. Pure culture studies of this endophyte may provide some informations about its nature and affinity

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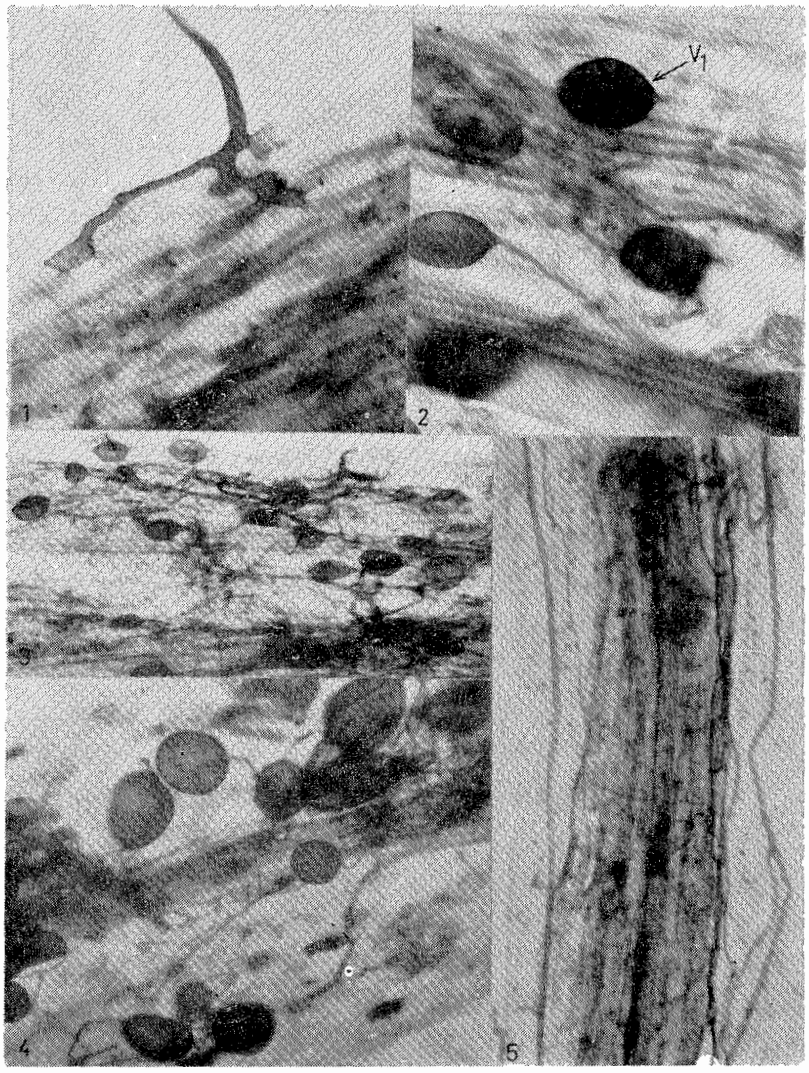

PLATE 1

Nos. 1—3 *Fagopyrum tataricum*

No. 1 Portion of the root with an appressorium. Note the hyphal branches arising from the appressorium X 100.

No. 2 Root cortex showing few versicles commonly found in roots infected with VA mycorrhizal fungus and one vesicles (VI) with reticulate divisions inside X400.

No. 3 Large number of vesicles and internal mycelium X 100

Nos. 4—5 *Plantago major*

No. 4 Portion of the root showing large number of vesicles and mycelium of mycorrhizal fungus X 150.

No. 5 Root showing hyphae of mycorrhizal fungus mostly present in the inner cortex X 100.

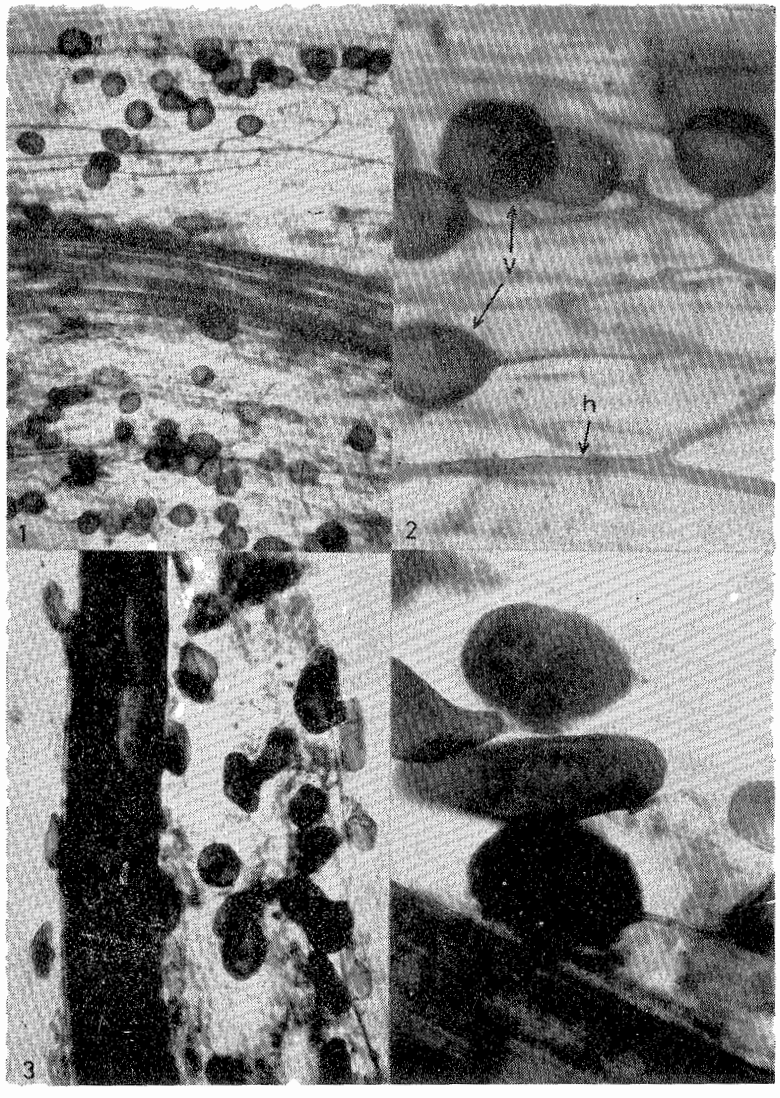

PLATE 2

Nos. 1 – 2 *Euphorbia falcata*.

No. 1 Portion of the root showing large number of vesicles in the cortex. Fungal hyphae traversing the cortex are also visible X 100.

No. 2 Portion of No. 1 at higher magnification. Fungal hyphae (h) giving off branches at the tips of which vesicles (v) are borne X 400.

Nos. 3—4 *Ranunculus* sp.

No. 3 Portion of root showing large number of vesicles which are characteristically large X 100.

No. 4 Vesicles in different form and at different stages of maturity in the root cortex X 450.

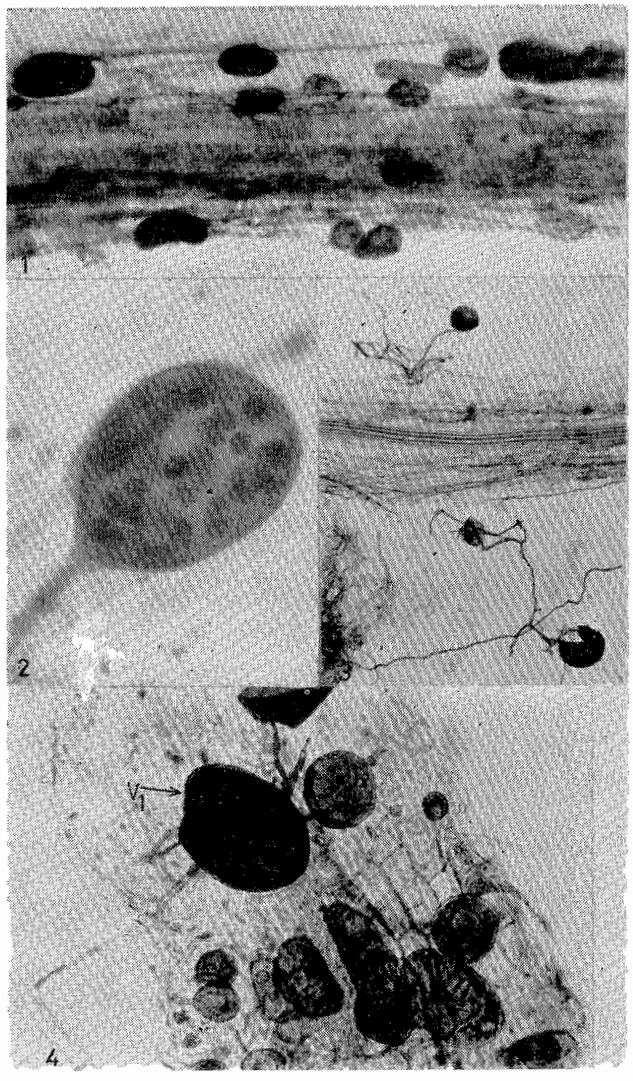

PLATE 3

Nos. 1—2 *Conyza canadensis*

No. 1 Portion of the roots showing large number of vesicles differing in size and maturity X 100.

No 2 A vesicle in the cortex having large number of rounded structures. The nature of the rounded structures inside the vesicle is not known X 450.

Nos. 3—4 *Nicotiana* sp.

No. 3 Portion of the root showing germinating Endogonaceous spores on the surface X 100.

No. 4 Cortical cells showing vesicles of various shapes X 100.

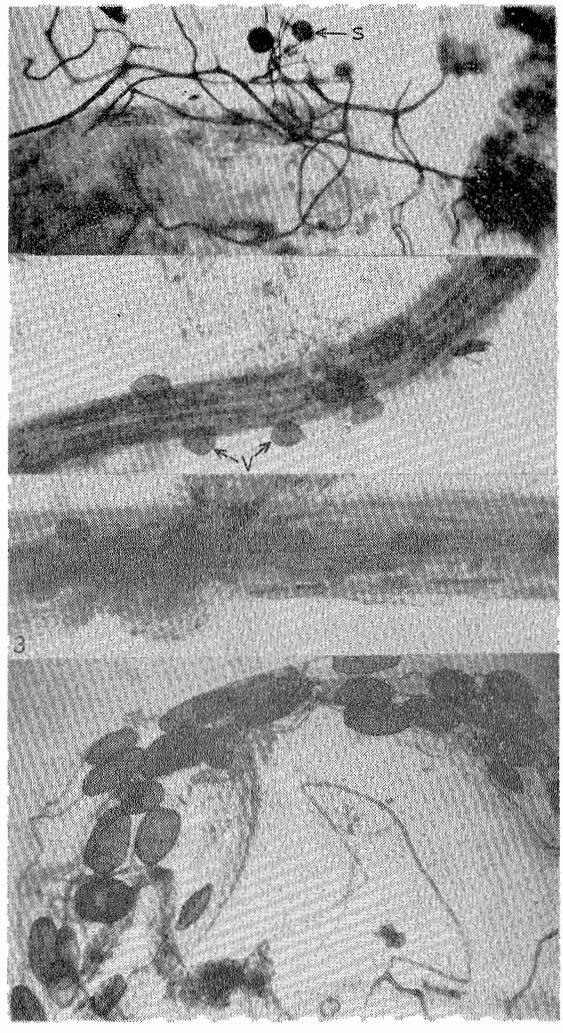

PLATE 4

No. 1 Portion of the root of *Rumax hastatus* showing large amount of external mycelium and Endogonaceous spores (s) X 100.

No. 2 Portion of root of *Sonchus arvensis* showing vesicles (v) in the cortex X 100.

No. 3 Root of *Chenopodium botrys* showing vesicular-arbuscular mycorrhizal infection X 100

No. 4 Portion of the root of *Hippurius vulgaris*, a member of Hippuridacene showing large elliptical to oval vesicles X 100.

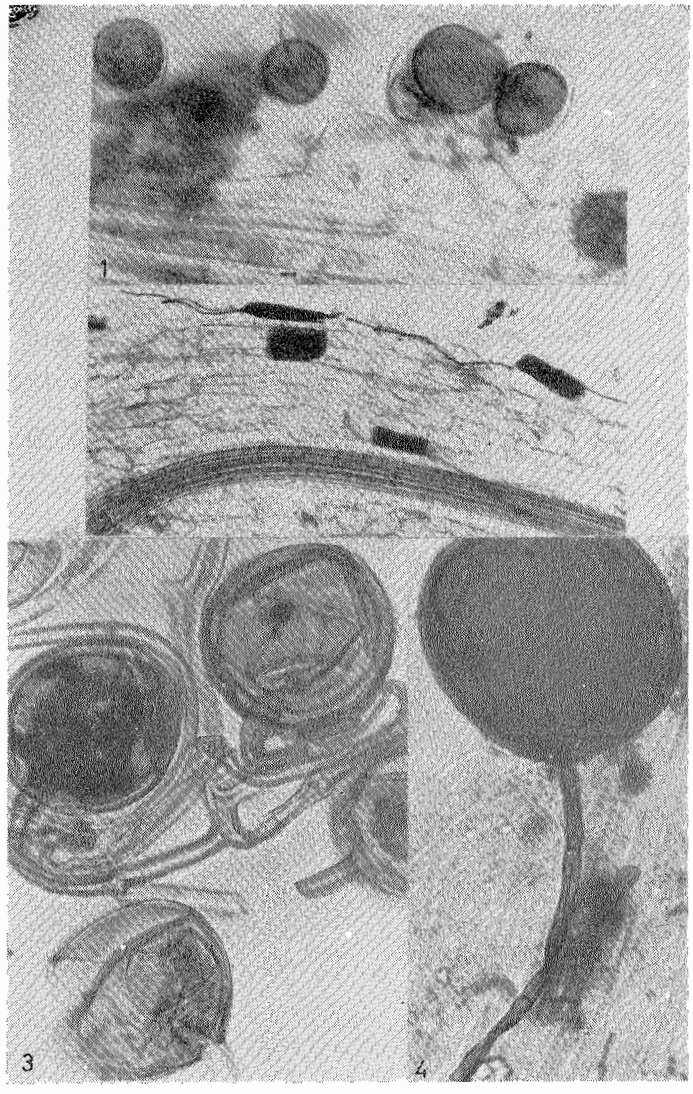

PLATE 5

Nos. 1—2 *Myriactis wallichi*.

No. 1 Portion of the root cortex showing vesicles X 100.

No. 2 Root cortex showing beaded structures with hyphal attachments X 100.

No. 3 Few spores of *Glomus mosseae* isolated from the rhizosphere of *Taraxacum officianale* X 400.

No. 4 Endogonaceous spore with septate subtending hypha X 400.