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Endogonaceous spore flora of Pakistan. IX. Frequency of occurrence of VAM fungi in wheat fields around Punjab University Campus area

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Abstract

For the screening of Endogonaceous spore types the rhizosphere soil was sampled randomly from three adjacent wheat fields around new University campus. Spore extraction was done following wet sieving and decanting technique and direct soil paste method. Decaying root pieces and sheathing leaf bases on decaying wheat stumps were also studied after staining.

A total of forty spore types were recorded from soil samples and decaying plant debris. Species of *Glomus* predominated the samples. There were twenty-eight species of *Glomus* recorded in the soil samples. One *Glomus* species i.e., *Glomus monosporum* formed sporocarps in the decaying sheathing leaf bases of left over wheat stumps after the crop was harvested. Four species of *Acaulospora*, three species of *Sclerocystis*, two species each of *Gigaspora* and *Scutellospora* were recorded. Among highly abundant species were *Glomus fasciculatum, G. mosseae, G. constrictum, G. aggregatum*, and *G. microaggregatum*.

Key words: Endogonaceous spores, VAM, wheat field, *Glomus*

Introduction

The most wide spread fungi in the rhizosphere of Angiosperms are Zygomycetous fungi that form symbiotic association with plant roots, the vesicular arbuscular mycorrhizal fungi (Kapulnic and Dauds, 2000; Mukerji *et al.*, 2000; Harley and Harley, 1987; Trappe, 1987). The fungal symbionts are geographically ubiquitous soil inhabitants, which must colonize plant roots to grow and reproduce. These have been found in diverse habitats ranging from the arctic to the tropics and to aquatic environment and stable plant communities to highly disturbed ecosystems. Ninety percent (90%) of the plants ranging from thallophytes to angiosperms has this association. This association is not restricted to the roots of plants only but is also found in all those organs of higher plants which are concerned with the absorption of substances from the soil (Srivastava *et al.*, 1996). However species of VAM fungi differ geographically in their frequency of occurrence and population density.

The present wide spread interest in vesicular arbuscular mycorrhizas makes it extremely important to define the properties of the individual fungal species or isolate precisely (Bowalda *et al.*, 1982). Selecting the right organism to help solve the right problem is further complicated by differences in plant responses not only to vesicular arbuscular mycorrhizal fungal species but also to their geographical isolates "edaphotypes" sensu Bethlenfalvy *et al.*, (1989).

Utilization of the endophytes in agricultural situations, therefore not only poses the problem of selecting one "best" organism but a collection of organisms which are compatible with each other, with native microflora with new host plants and with the new host soil (Dagoberto *et al.*, 1986; Hayman, 1987).

The purpose of the present study is to enlist and characterize VAM forming fungi from wheat fields around Quaid-e-Azam Campus, University of the Punjab, Lahore, Pakistan.

Materials and Methods

Sampling was carried out in transacts in three adjacent fields. Twenty samples of approximately 100g soil per sample of root zone and rhizosphere were collected per field, then mixed to give ten final samples per field.

Endogonaceous spores were extracted by the wet sieving and decanting technique of Gerdemann and Nicolson (1963), and also by a direct soil paste method (Nasim and Iqbal 1991). In the former 100g of soil was soaked in 1000 ml of water for 24 hours. The supernatant was then passed through a gradient of sieves with pore sizes ranging from 400um to 50um arranged one above the other in an ascending order. Each sieve was then washed in sterilised water and filtered through Whattman No. 1 filter paper. This filter

was then observed under a microscope for the presence of various kinds of Endogonaceous spores.

In the later method soil was spread into a thick paste and spores were directly picked up with the help of a sharpened toothpick under a dissecting microscope. The soil samples were thoroughly screened for Endogonaceous spore types.

Spores were either picked up with a sharpened toothpick or hypodermal needle was also used for this purpose. After washing several times, spores were either mounted in a drop of sterile water or these were stained in a drop of trypan blue in lactophenol and examined under the microscope.

Decaying root pieces and plant debris were also picked up observed under the microscope after giving several washing with sterile water. The plant portions were stained following the method of Phillips and Hayman (1970) with some modifications (Iqbal and Nasim, 1986).

Spores and other structures were microphotographed with the help of Minolta X700 with an adapter tube.

Results

The percentage frequency of occurrence of vesicular arbuscular mycorrhizal fungal propagules per 100g of rhizosphere soil sample was highly variable. Endophytes like *Glomus constrictum, G. fasciculatum, G. mosseae, G. microcarpum* and *G. microaggregatum* were highly abundant, while *Gigaspora pellucida*, some unidentified species of *Glomus*, *Glomus halonatum*, *G. intraradices* and *G. tenui* were of rare occurrence. Rest of the species showed intermediate values for the frequency of occurrence, (Table 1& 2). It is also evident from the results that most of VAM fungi forming spores in association with wheat belong to the genus *Glomus* (Morton, 1988). The existence of *Glomus* as the dominant genus in the root zone indicates either the influence of the soil or the plant type (Schenck & Kinlock, 1980). These results are also in line with those of Nasim & Iqbal (1991b) and Iqbal & Nasim (1991).

In the present study there are 28 species, which belong to genus *Glomus*. It indicates that about 70% of the VAM species composition is made by the genus *Glomus* in this very typical cropland ecosystem. There were 7 species of *Glomus*, which remained unidentified, but their identity as species of the genus *Glomus* was beyond any doubt. The genera *Acaulospora, Sclerocystis, Gigaspora* and *Scutellospora* accounted for the rest of the percentage represented by 4, 3, 2, and 2 species respectively. One species each of *Acaulospora, Gigaspora* and *Scutellospora* also needed to be identified up to species level. Thus a total of about 25% of the species could not be identified up to species level (Table 1& 2).

Table 1: Endogonaceous spore flora of wheat fields at Quaid-e-Azam Campus, University of the Punjab, Lahore, Pakistan.

Sr. No.	Species of AMF	%age of spores per 100 g soil
1.	*Acaulospora bireticulata* Rothwell & Trappe	+ + +
2.	*A. foveata* Trappe & Janos	++
3.	*A. rehmii* Sieverding & Taro	++
4.	*Acaulospora sp.*	++
5.	*Gigaspora decipiens* Hall & Abbott	+
6.	*Gigaspora sp.*	++
7.	*Glomus aggregatum* Schenck & Smith emend.Koske	++++*
8.	*G. albidum* Walker & Rhodes	++
9.	*G. caledonicum* (Nicol. & Gerd.) Trappe & Gerd.	+++
10.	*G. cerebriformis* Mc Gee	++
11.	*G. clarum* Nicolson & Schenck	++
12.	*G. constrictum* Trappe	++++*
13.	*G. dilhiense* Mukerjee, Bhattacharjee & Tewari	+++
14.	*G. deserticola* Trappe, Bloss & Tewari	+++
15.	*G. dimorphicum* Boyetchko & Tewari	++
16.	*G. fasciculatum* (Thaxture) Gerd. & Trappe emend. Walker & Koske	++++*
17.	*G. ficundisporum* Schenck & Smith	+++
18.	*G. geosporum* (Nicol. & Gerd.) Walker	++
19.	*G. halonatum* Ross & Trappe	+

20.	*G. intraradices* Schenck & Smith	+
21.	*G. leptotichum* Schenck & Smith	++
22.	*G. microaggregatum* Koske, Gemma & Olexia.	++++*
23.	*G. microcarpum* Tul. & Tul.	++
24.	*G. monosporum* Gerd. & Trappe	+++
25.	*G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe	++++*
26.	*G. multicaul* Gerd. & Bakshi	++
27.	*G. reticulatum* Bhattacharjee & Mukerji	+
28.	*G. tenuis* (Greenall) Hall	+
29.	*Glomus sp.* I	++
30.	*Glomus sp.* II	+
31.	*Glomus sp.* III	+
32.	*Glomus sp.* IV	++
33.	*Glomus sp.* V	++
34.	*Glomus sp.* VI	++
35.	*Glomus sp.* VII	+
36.	*Sclerocystis micrcarpus* Iqbal & Bushra	++
37.	*S. pakistanica* Iqbal & Bushra	+++
38.	*S. sinuosa* Gerd. & Bakshi	++
39.	*Scutellospors pellucida* (Nicol. & Schenck) Walker & Sanders	++
40.	*S. verricosa* (Koske & Walker) Walker & Sanders	++

Key: + = 0-25%, ++ =25-50%, +++ = 50-75% & ++++ = 75-100%. * = highly abundant species

Table 2: Details of infections and spore characteristics

Spores of VAMF	Plate/fig. No.	Details of infections
Glomus monosporum	(Plate1B) (Plate 4D-F)	Sporocarps of *G. monosporum* were found in the soil as well as in the decaying sheathing leaf bases and roots. In soil these were mostly encountered in the rhizosphere of decaying stumps, while the sporocarps develop and mature in these stumps (Nasim & Zahoor, 1997). In most of the sporocarps only one spore was observed but occasionally 2-3 spores per sporocarps were also seen. Oil droplets were also observed in the spores. Spores immediately popped out when a slight pressure was applied on the cover slip. Spores of *Glomus monosporum* ranging in colour from yellow to brown (140-330μm in diameter) were observed with 100% frequency of occurrence in most of the soil samples. Some spore like vesicles were 40-50 μm in diameter with smooth outer wall (4-10μm thick), clear inner contents and subtending hyphae. Subtending hyphae were very delicate and branched slightly recurved in some specimens. The spores were present in soil mostly adhering to leaf bases in soil samples.
Acaulospora rehmii	(Plate 1C)	Brown coloured at low power but at high power (40x) of the microscope the spores (82-)112-168(-175) μm appeared yellowish brown. The outer spore wall was not smooth and had a pattern of dark brown reticulations on the surface. This resembles a channel system. Number of wall grpups were 3, 4.5-17μm thick. Spores were sessile without any subtending hypha.
Glomus aggregatum	(Plate 1D)	Spores formed singly or in clusters in the rhizosphere of wheat. Spores were yellow to brown in colour having a size of (20-40-85 (-120) μm. Most of the spores were encountered with thin hyphal connections. These were thin walled (1-2 wall groups with a thickness of 2-6(-10) μm) and were of various shapes. Some spores appeared dead due to the presence of eroded outer walls and bacterial invaginations (Nasim & Iqbal, 1991). Some spores were seen inhabited by other spore forming fungi. In some specimen however, the invading spores were probably formed due to internal proliferation.

Glomus aggregatum	(Plate 3B)	This species was found in the form of loose aggregates in association with the decaying vascular bundles of roots of wheat. The spores were brown , yellowish brown in colour. Average size of the spores was 60-80μm in diameter. Spores were globose to sub-globose, double walled and walls were separable on applying slight pressure on the cover slip. Spores were found having a straight or recurved hyphal attachment. This species of VAM fungi was recorded from almost all the soil samples.
Glomus sp. 1 and 2	(Plate 1E & F)	Many spores belonging to the genus *Glomus* were also observed but their species identity could not be ascertained. The material has been sent to the authorities in United States of America and Canada for identification. Spores of *Glomus* species 1 were dark coloured with thick walls and slightly bulbous subtending hypha. It was found in association with decaying vascular bundles of wheat roots. Spores of *Glomus* species 2 had very thick walls. These were sub-hyaline and sessile.
Acaulospora faveota	(Plate 1G)	The frequency of this spore was up to 50% (Table 1). Spore was sessile, ellipsoid or globose and light reddish brown in colour. Spore surface was uniformly pitted with mostly rounded depressions. Spores are born laterally on a hyaline, thin-walled funnel like hypha ±75μm in diameter at the point of spore attachment.
Glomus mosseae	(Plate 2A)	These spores were recorded in all samples of rhizosphere soil. This was one of the highly abundant species of spores found in association with wheat. Spores which had a size of 105-310x110-305μm, were found singly or in aggregates. They ranged from light yellow to brown in colour and globose to ovoid in shape. Many of the spores in a cluster were seen with air bubble trapped inside them. Spores were with single outer wall which was 2-7μm thick.
Glomus sp. 3	(Plate 2B)	The sporocarps of this unidentified *Glomus* species were also recorded in some samples. In samples collected at the end of wheat season, the %age frequency of occurrence in the samples was 100%. The sporocarp wall or peridium appeared to be very loosely interwoven and light yellow in colour. The spores inside the peridium were dark reddish brown or brown in colour. Spore walls were not smooth.
Glomus geosporum	(Plate 2C)	Light lemon to black coloured and globose in shape and 110-290x100-290μm in size, were found usually with hyphal attachments. The spores were single walled (4-18μm thick). The subtending hypha was straight in most of the spores while in some spores it was slightly funnel shaped. In some spores inner contents appeared to be granular.
Glomus sp. 4	(Plate 2D)	Black coloured sporocarps of *Glomus* species were observed in some samples. The peridium or the spore wall appeared to be very thick and hard but at some places network of hyphae was also noticed. The %age of occurrence of these propagules was 25%.
Glomus sp. 5	(Plate 2E)	Another sporocarp of an unidentified *Glomus* species was encountered in the soil samples and the %age frequency of occurrence of this species was 50%. The peridium was very loosely woven and inconspicuous and hyaline. The spores inside the sporocarp were light brown to brown in colour. The spore wall appeared to be very fragile and delicate. The spores were thin walled. In some specimens the spores seemed to have double walls, with an inner wall thicker than outer wall. The percentage frequency occurrence of these sporocarpic species was 100% in processed plant materials like roots and sheathing leaf bases collected after crop harvest.
Scutellospora pellucida	(Plate 2F)	The percentage frequency of occurrence of this species was 25% only. These spores were in very few samples. Spores were encountered singly in soil samples, globose in shape (58-183(-250) μm in diameter) and hyaline to brown in colour. The spores were double walled with outer

		wall fractured at certain points. The suspensor (10-29(-50) μm wide) like subtending hypha was swollen at the point of attachment was even lighter in colour than the spore itself. Septum separating the subtending hypha from the spore was clearly seen.
Glomus leptotichum	(Plate 2G)	These spores were found singly in soil, irregular and regular in shape and size varied from 110-250μm to 150-175μm. The percentage occurrence of spores was 50%. Spores were single walled, creamy brown in colour and spore wall continuous with the subtending hypha. Spore wall appeared to be granulated, with small ridges which were prominent near the subtending hypha. Subtending hypha wider (10-15 μm in diameter) at the point of attachment narrowing gradually (5-6μm in diameter) in the distal part.
Glomus retriculatum	(Plate 3A)	A lose cluster of spores was observed in the collections having a percentage frequency of occurrence of 50%. The spores were yellowish brown in colour with reticulation on the spore surface. Average size of the spore was 130-170μm in diameter. Spores appeared to be double walled which were 10-15μm thick. Outer wall with reticulations on the surface and the inner wall smooth.
Glomus caledonium	(Plate 3C)	Spores found singly in soil, light yellow to brown in colour and got stained very quickly in trypan blue stain. Spores were large in size about 120-279μm in diameter, double walled, 6-109-16) μm thick. Outer wall thicker at the point of hyphal attachment, inner wall extended into the hyphal attachment a short distance. Clear and transparent spore contents were separated from the subtending hypha by the presence of a septum in the subtending hypha.
Polygonal spores	(Plate 3D)	These polygonal spore like bodies were clearly observed in stained roots. These senescing roots were collected after crop harvest. These were arranged in rows and were darkly stained.
Acaulospora sp.	(Plate 3 E)	Reddish brown spores with a peculiar pattern on the walls were observed with a frequency of 50%. Irregular or pear shaped with average size of 130-175μm in diameter. Subtending hypha stained quickly in trypan blue. Spores were born on a colour less main hypha with septation collected from the rhizosphere soil.
Glomus sp. occupied by some other *Glomus* sp.	(Plate 3F)	An unidentified *Glomus* species recovered from the soil samples was inhabited by some other *Glomus* species. Occupied spores were light brown in colour and stained in trypan blue. Spores were double walled and 125 μm in diameter. Occupants were dark brown in colour, 40-50μm in diameter, globose to sub-globose and double walled. Inner wall was thicker and smooth and outer wall was comparatively thin and rough in appearance.
Gigaspora sp.	(Plate 4A)	A cluster of spores of *Gigaspora* sp. showing 50% frequency of occurrence was collected from some soil samples. Spores were in aggregates, lemon yellow in colour, average size 50-70μm in diameter, globose and sub-globose in out line. Subtending hypha swollen at the point of attachment with the spore. The diameter of the subtending hypha decreased in the distal part.
Glomus microaggregatum occupied by *Glomus tenui* in a *Glomus* sp.	(Plate 4B)	An unidentified *Glomus* species observed in a soil sample was found occupied by *Glomus microaggregatum* which in turn was inhabited by *Glomus tenui*. The unidentified *Glomus* sp. was thick walled and globose in outline.
Sclerocystis pakistanica	(Fig. 1)	*Sclerocystis pakistanica* had 75% frequency of occurrence with smooth thin walled (2μm) spores but walls slightly thicker (4μm) at the base. Sporocarp ranged in size from 520-590(-700)μm with thick peridium. The spores were 65-205x33-55μm in size. These spores occupied by some *Glomus* sp. were also observed frequently. The *Glomus* species was identified as *G. microaggregatum* which are light coloured double

		walled, walls being very thin with colourless subtending hyphae. The size of the spore of *G. microaggregatum* was 30-40μm in diameter and shape was globose to sub-globose. Some spores were irregular due to the pressure of inner walls of *Sclerocystis pakistanica.*
Sclerocystis sinuosa	(Fig. 2)	Sporocarps of *S. sinuosa* were observed in some specimens. Sporocarps were globose in outline and their average size was 248-272μm in diameter. Sporocarps wall was made up of sinuous hyphae gave a characteristic appearance to the sporocarps and distinguished these from rest of the *Sclerocystis* species. Spore were ranging in size 45-118x30-83μm.
Sclerocystis micrcarpus	(Fig. 3)	Sporocarps were dark brown in colour with tough outer peridium and were 100-420μm in diameter. Chlamydospores (95-115x40-60μm) were clavate and cylindrical dark brown in colour and with laminate walls much thicker (9-26μm) at the apex of spores.
Senescing leaf bases	(Plate 4C)	Senescing leaf bases of wheat were collected from the field soil. These were stained following the usual procedures. These leaf bases were heavily mycorrhizal. Mycelial and vesicular infections were 100%. Vesicles were darkly stained and were of different shapes and sizes. Most of the vesicles were with hyphal attachments.

Discussion

Arbuscular fungi are obligate biotrophs that are unable to sustain growth and reproduction apart from a plant host. This restrictive niche appears to be balanced by an extremely wide host range, so that ample opportunities exist for any dispersed fungal propagule to establish a new individual somewhere else. The filamentous habit characteristic of this and other fungi affords organisms considerable versatility in their life cycles, with the capability of indefinite growth as long as carbon from a host plant source is available. All indications are that reproduction is clonal and that spores formed asynchronously as mycorrhizal colonization progresses are somatic offshoots compartmentalizing a varying number of nuclei (Kapulnik and Douds, Jr., 2000)

The percentage frequency of occurrence of vesicular arbuscular mycorrhizal fungal propagules per 100g of rhizosphere soil sample was highly variable. Endophytes like *Glomus constrictum, G. fasciculatum, G. mosseae, G. microaggregatum* and *G. microaggregatum* were highly abundant, while *Gigaspora pellucida*, some unidentified species of *Glomus*, *Glomus halonatum, G. intraradices* and *G. tenui* were of rare occurrence. Rest of the species showed intermediate values for the frequency of occurrence, (Table 1 & 2). It is also evident from the results that most of VAM fungi forming spores in association with wheat belong to the genus *Glomus* (Morton, 1988). The existence of *Glomus* as the dominant genus in the root zone indicates either the influence of the soil or the plant type (Schenck and Kinlock, 1980). These results are also in line with those of Nasim and Iqbal (1991b) and Iqbal and Nasim (1991).

The classification of the Glomales is largely based on the structure of their soil-borne resting spores, but more recently the careful study of developmental processes and biochemical properties have provided valuable information (Walker, 1992; Morton, 1993). Accurate identification of Glomalean fungi often requires them to be isolated in cultures with host plants, to observe developmental stages and avoid the loss of diagnostic features which occurs in field-collected material. The fungi that form AM are currently all classified in the order Glomales (Morton, 1988). The taxonomy is further divided into suborders based on the presence of: (i) vesicles in the root and formation of chlamydospores (thick wall, asexual spore) borne from subtending hyphae for the suborder Glomineae or (ii) absence of vesicles in the root and formation of auxiliary cells and azygospores (spores resembling a zygospore but developing asexually from a subtending hypha resulting in a distinct bulbous attachment) in the soil for the suborder Gigasporineae. The AM type of symbiosis is very common as the fungi involved can colonize a vast taxonomic range of both herbaceous and woody plants, indicating a general lack of host specificity among this type.

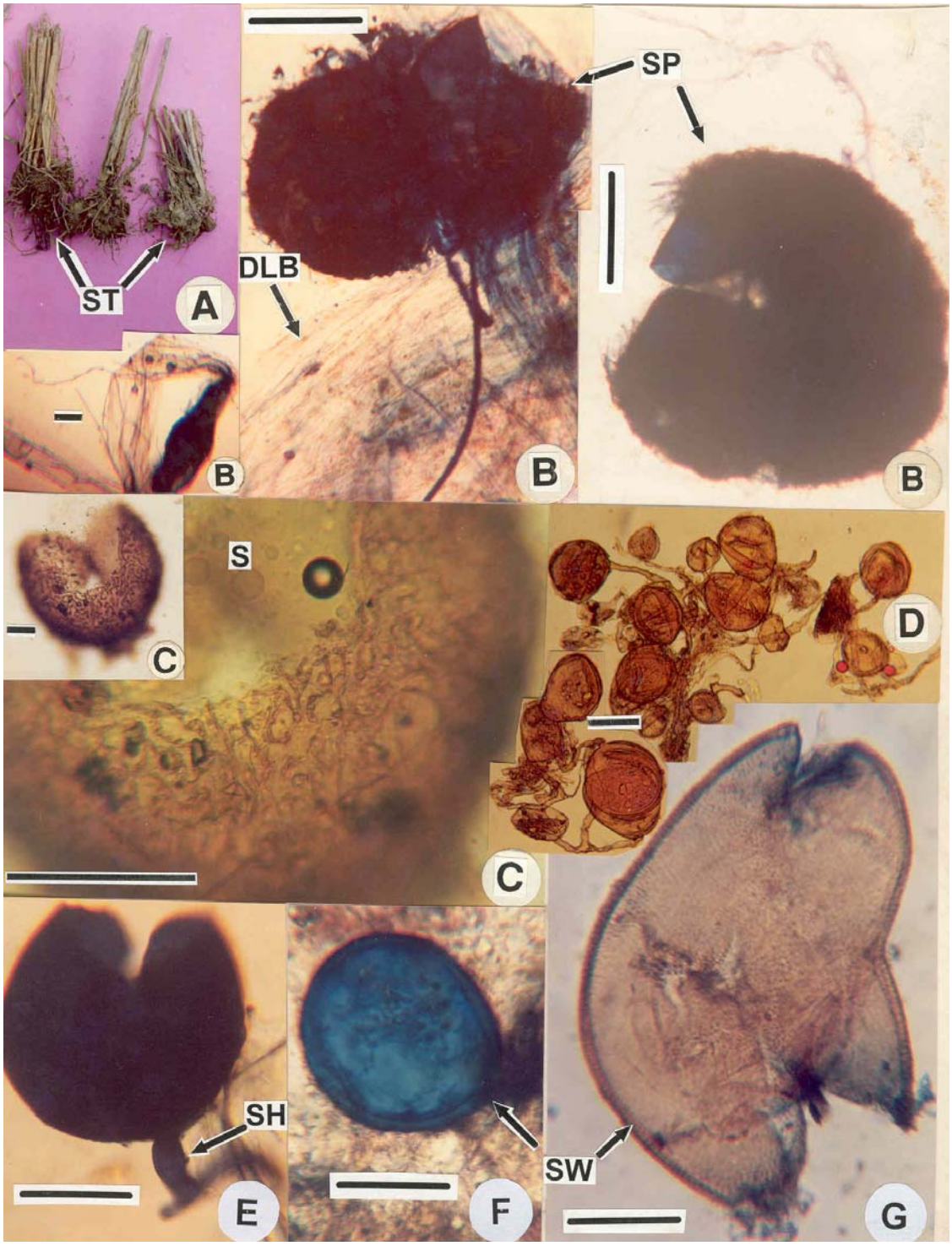

Plate: 1 A: Wheat stumps taken from a cultivated wheat field after harvest.
B: Sporocarps of *Golmus monosporum.*
C: *Acaulospora rehmii.*
D: A cluster of spores of *G. aggregatum.*
E: *Glomus* sp.
F: *Glomus* sp., thick walled spores.
G: *Acaulospora foveata*.
Key: ST: stumps, SP: sporocarp, DLB: decaying leaf bases, SW: spore wall, SH: subtending hypha.

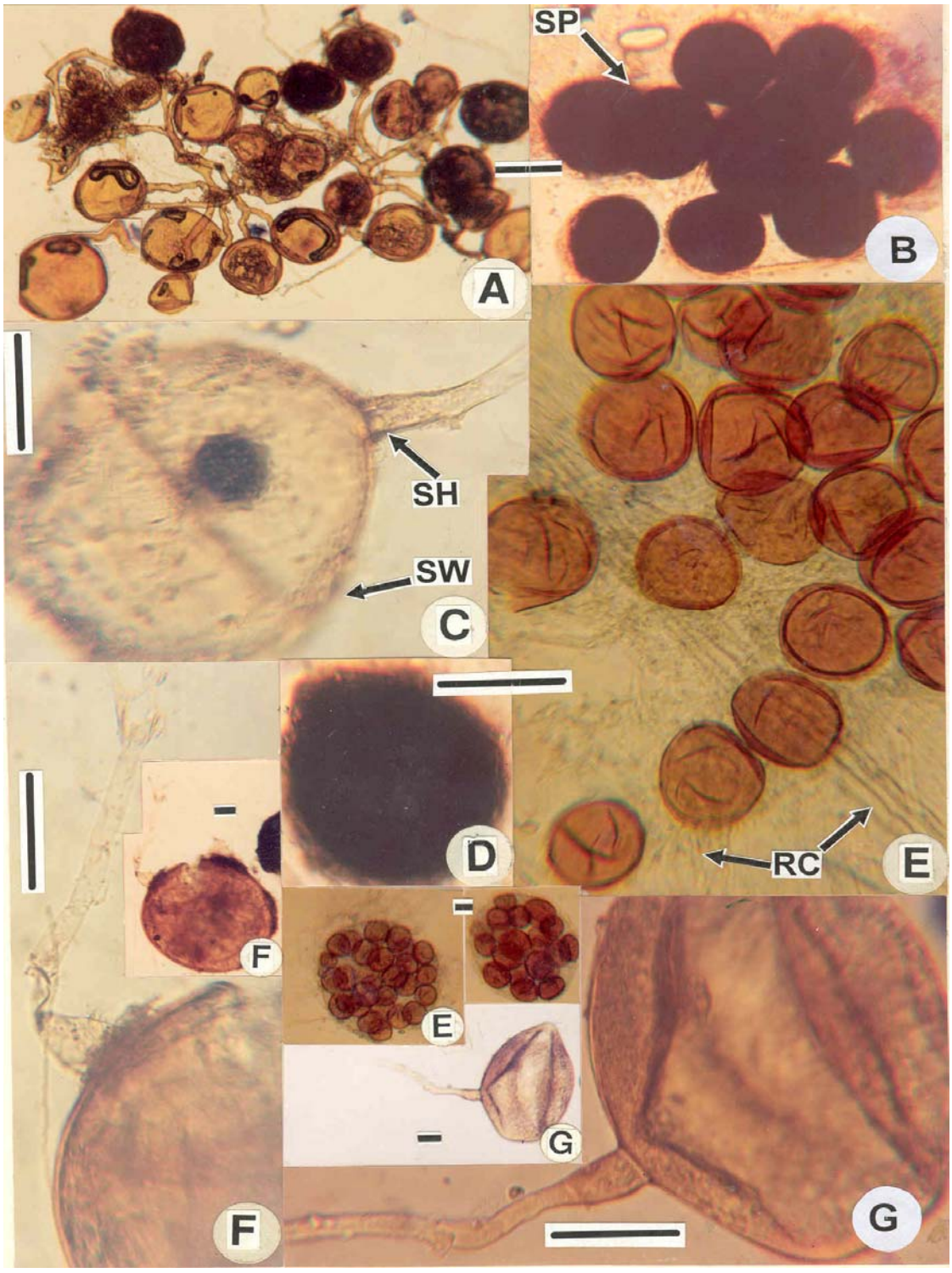

Plate: 2 A: A cluster of spores of *Glomus mosseae.*
B: Sporocarp of some *Glomus* sp.
C: *Glomus geosporum.*
D: Sporocarp of an unidentified *Glomus* sp.
E: Sporocarp of *Glomus* sp.
F: *Gigaspora pellucida.*
G: Spores of *Glomus leptotichum.*
Key: S: spore, SW: spore wall, RC: root cortex, SP: sporocarp, SH: subtending hypha.

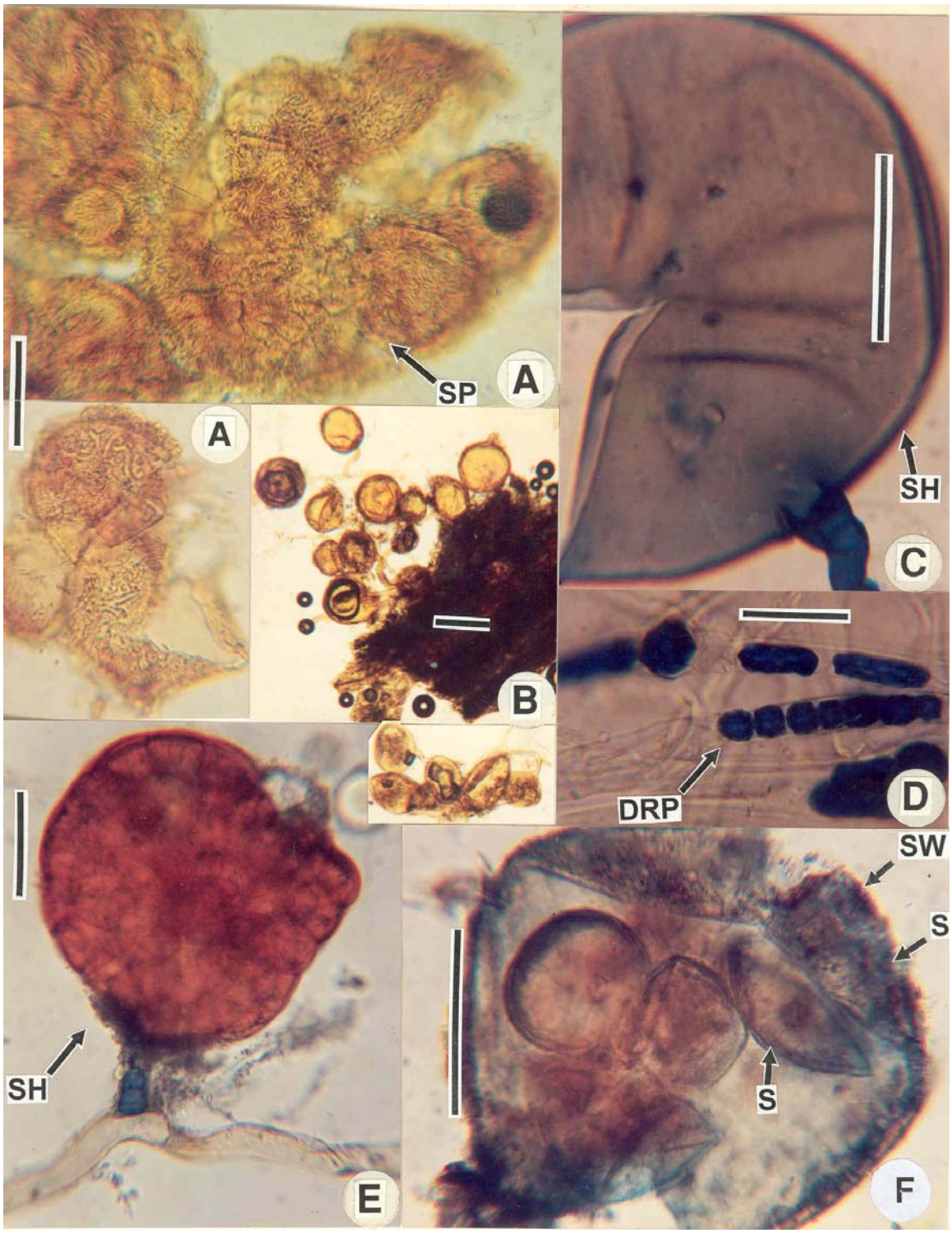

Plate: 3A: An aggregate of spores of *Glomus reticulus* Spores showing reticulations on surface.
B: Spores of *G. aggregatum* associated with the vascular bundle of a dried root.
C: *Glomus caledonicus.*
D: Polygonal squarish and elongated spores in wheat roots.
E: *Acaulospora* sp.
F; *Glomus* sp. Occupied by the spores of some other *Glomus* sp.
Key: SP: sporocarp, S: spore, SH: subtending hypha, DRP: decaying root piece.

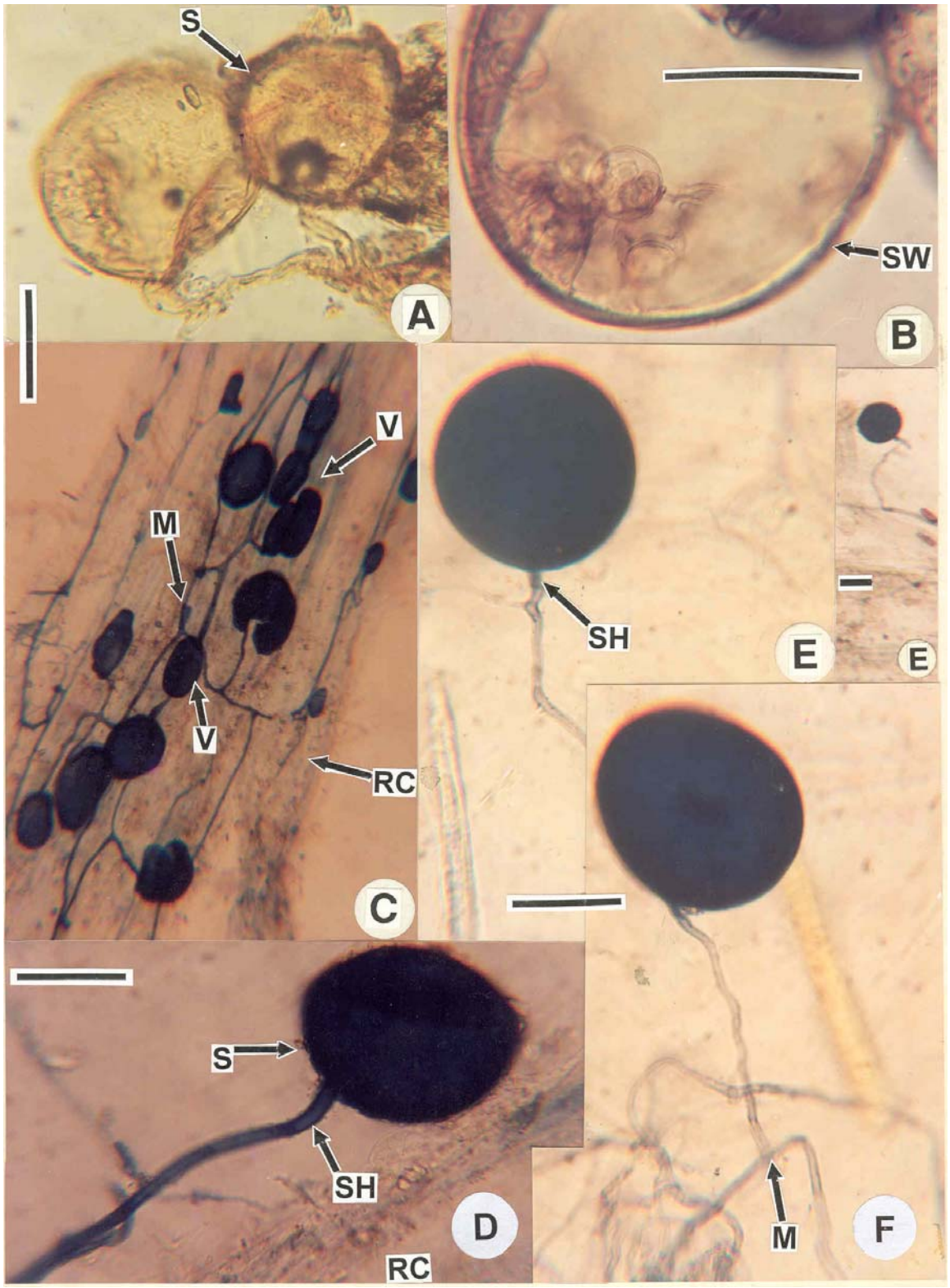

Plate: 4A: *Gigaspora* sp.
B: *Glomus microaggregatum* occupied by *G. tenui* in a *Glomus* sp.
C: Oblong vesicles with hyphal attachments in a leaf base.
D-F: *Glomus monosporum* with subtending hyphae.
Key: S: spore, M: mycelium, V: vesicles; RC: root cortex, SW: spore wall, SH: subtending hyphae.

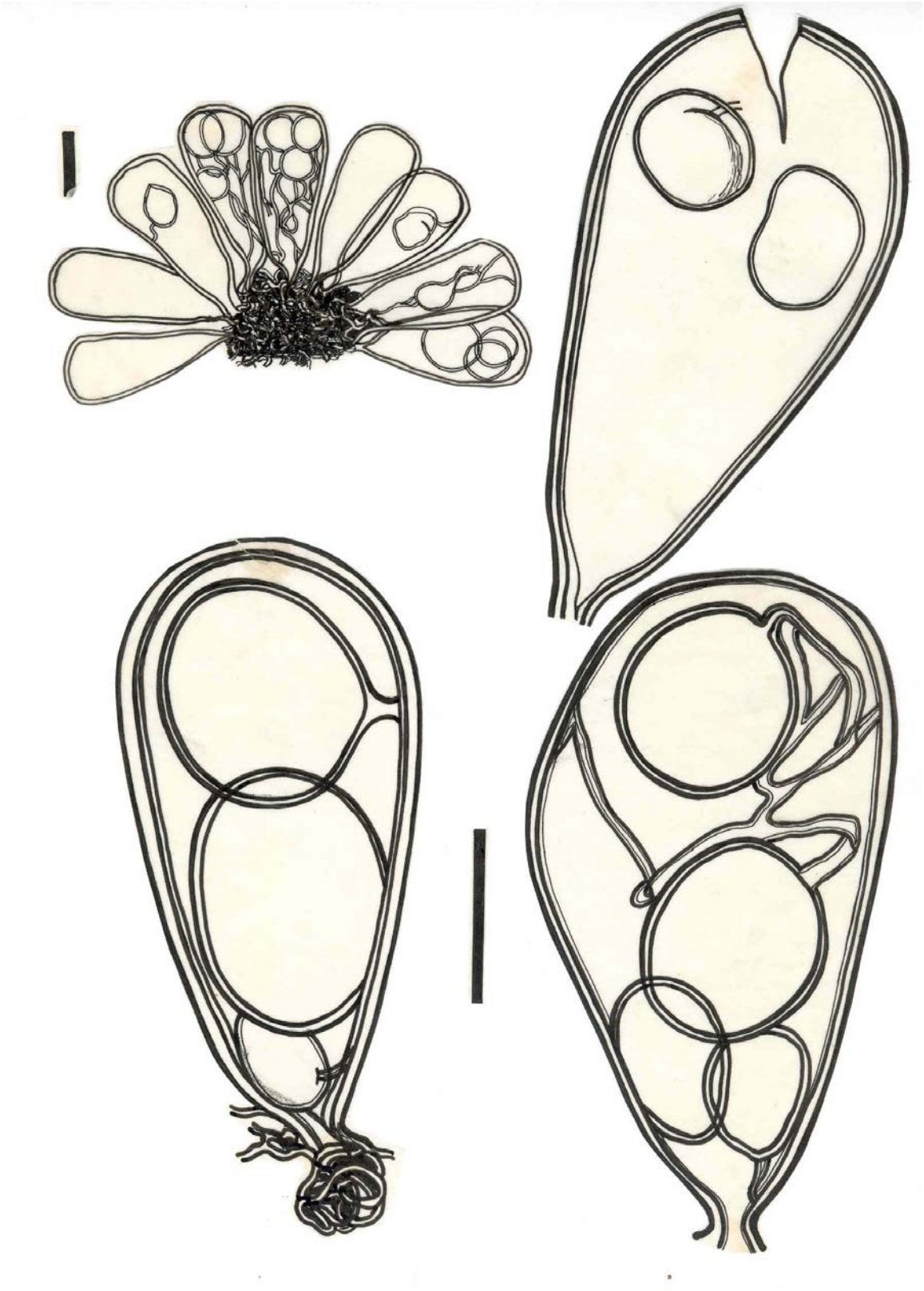

Figure-1: Spores of *Glomus microaggregatum* inside spore of *Sclerocystis pakistanica*.

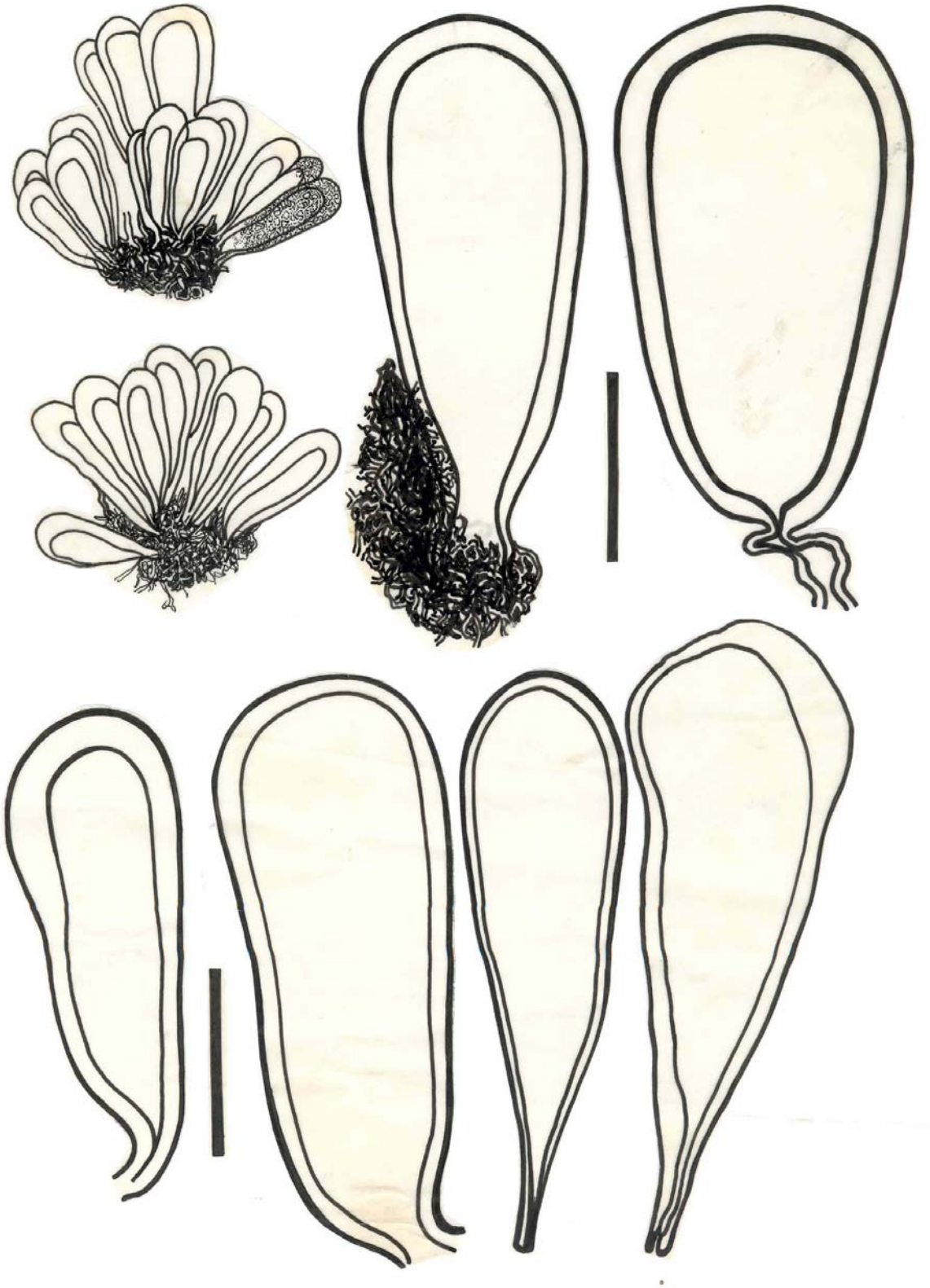

Figure-2: Spores of *Sclerocystis sinuosa.*

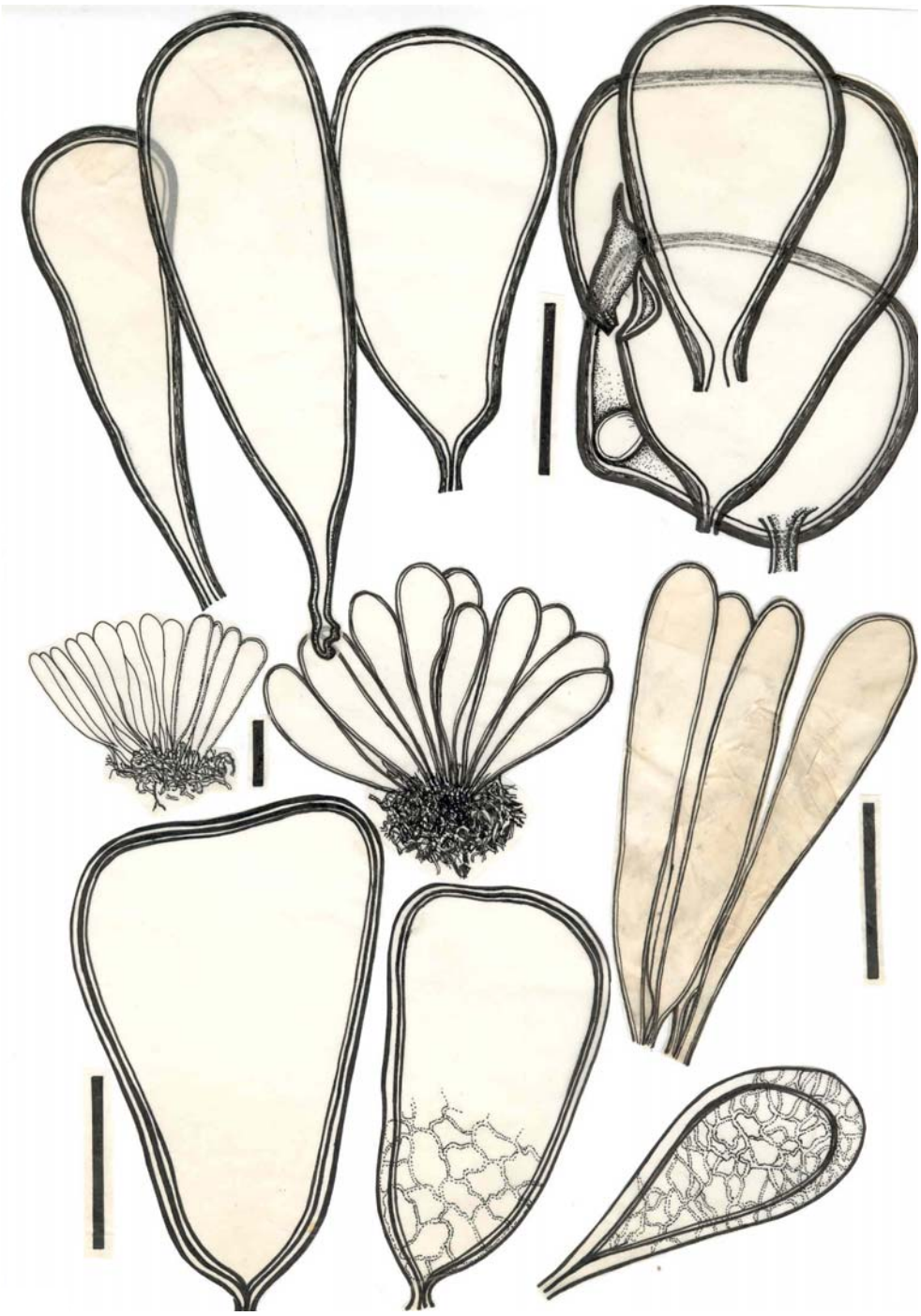

Figure-3: Spores of *S. macrocarpus* (bar=50μm).

Vesicular arbuscular mycorrhizal fungi are an intimate component of the soil in all types of environments and there is an apparent lack of host specificity among these fungi (Smith and Read 1997). Therefor in the present study a wide variety of VAM fungi have been reported in the vicinity of wheat roots. However, the types of fungi sporulating in the sheathing leaf bases are restricted. It may be due to the qualitative and quantitative nature of the exudates from the later (Potty, 1985). The plant portions like these may thus be exploited for the mass multiplication of specific species of VAM (Nasim and Iqbal, 1991).

The present study was under taken to collect spores of VAM fungi for identification of indigenous species associated with wheat crop and

to establish pot cultures of prominent species. The goal of this work is to eventually inoculate plants in the field in Pakistan with superior strains and to introduce other known species from other parts of the world that might also be effective.

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