

ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH TISSUE CULTURE RAISED POTATO

GHAZALA NASIM

Institute of Plant Pathology

University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan.

E. mail: ghazalanasim@hotmail.com

Abstract

In the present paper the types of arbuscular mycorrhizal propagules in the rhizosphere soil of the four different varieties of potato raised by meristem culturing method have been enlisted. A total of 29 species of Glomeralean spores were recorded in the soil. Nineteen of these spores associated with potato crop were reported in the genus *Glomus* while two species each of *Aculospora* and *Scutellospora* and one each of *Gigaspora* and *Sclerocystis* were recorded. Four species could not be identified with the help of available diagnostic keys. *Glomus mosseae* and *G. fasciculatum* were scored having highest prevalence in the rhizosphere of all the progenies of four varieties studied.

Introduction

The group of fungi forming arbuscular mycorrhizae are now being placed in Glomeromycota. This is a small group comprising of seven genera and 160 species. The genus *Glomus* comprising of 110 species is the most important genus of order Glomerales (Schüßler *et al.*, 2001). However the fungi are ubiquitous in nature and are world wide in distribution forming the mutualistic symbiotic association with over 95% of the plant species (Krishna, 2005; Johnson *et al.*, 2006). During the last 20 years, these fungi are reported to be of much wider occurrence than were thought of 3-4 decades ago. The literature is ample confirming their presence in portions other than roots (Nasim & Iqbal 1991a,b; Iqbal & Nasim, 1991). Colonization of roots by mycorrhizal fungi has been shown to improve growth and productivity of several field crops (Javaid *et al.*, 1993; Cavagnaro *et al.*, 2006) by increasing nutrient element uptake (Al-Karaki, 2002). They also impart other benefits to plants including production of certain secondary metabolites (Schliemann *et al.*, 2008), increased rate of photosynthesis (Wu & Xia, 2006), enhancement of nitrogen fixation by symbiotic or associative N₂-fixing bacteria (Javaid *et al.*, 1994), osmotic adjustment under drought stress (Auge *et al.*, 2008; Ruiz-Lozano, 2003), increased resistance to pests (Khaosaad *et al.*, 2007), tolerance to various abiotic stress factors (Takeda *et al.*, 2007), and improving soil aggregation (Wu *et al.*, 2008) and thus improved soil physical properties and stability. Studies have shown that AM fungi alleviate allelopathic stress and improve crop growth and yield (Bajwa *et al.*, 1999, 2003; Javaid 2007).

The significance of AM fungi for a tissue culture raised plantlets is of special concern. The plantlets mass multiplied through the aseptic technique have to face vicissitudes of the environment when are transplanted in the unsterilized field soil. Root borne plant diseases are one of them. Over 50% plantlet mortality have been reported at this stage. This hampers the utility of this technology for human well being. The plant tissue culture experts have been using chemicals for acclimatization of these fragile plantlets (Short, 1990; 1991). In view of the escalating cost of chemicals like antitranspirants & synthetic waxes and their unfriendliness to the environment, AM

fungal preinoculation may serve as a vital tool to reduce the mortality percentage. However to fulfill the objective, selection/s of the right organism or consortia of microbes is needed to be done carefully.

In the present paper, the fungi forming AM with four tissue culture raised potato varieties have been listed. The spore number in the rhizosphere of these potato varieties have been recorded so that the suitable associative fungi may be selected carefully. Therefore the major objective of the present study was to indicate and characterize AM fungus or a group of fungi potentially beneficial for the crop of tissue culture raised plantlets in potato growing area.

Materials and Methods

Sampling of rhizosphere soil was carried out in transects in three adjacent fields of 4 different varieties of potato viz., Desiree, Diamont, Cardinal and Patrones. The varieties were raised through meristem tip culturing (Fig. 1) on Murashige & Skoog's medium (1962) in potato tissue culture lab, Punjab Seed Corporation, Sahiwal, Pakistan. Four progenies such as 1st, 2nd, 3rd and 4th (Fig. 2) of the above mentioned varieties were sown in the experimental fields. Twenty samples of approximately 100g soil sample of root zone and rhizosphere were collected per field, then mixed to give ten final samples per field.

Glomalean spores were extracted by the wet sieving and decanting technique of Gerdemann & Nicolson (1963), density gradient centrifugation protocols (Daniels & Skipper, 1982; Furlan *et al.*, 1980) and also by direct soil paste method (Nasim & Iqbal, 1991a & b). In wet sieving and decanting technique, 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 400 μ m to 50 μ m arranged one above the other in an ascending order. Each sieve was then washed in sterilized water and filtered through gridded Whattman No. 1 filter paper. This filter paper was then observed under a microscope for the presence of various kinds of endogonaceous spores. Identification was done following Trappe (1982), Morton (1988) and Schenck & Perez (1987). The density was determined by counting the number of spores randomly in 5 squares and multiplied by the total number of squares on the filter paper.

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

In Sucrose centrifugation spores with minimal amount of organic particles were further purified by suspending sieving in 40% -60% sucrose solution and centrifuged at 2000 rpm for one minute. The supernatant (with spores) was assessed through sieves of 250 and 300 mesh and rinsed with distilled water to remove sucrose (Daniels & Skipper, 1982).

In Glycerol centrifugation spores were also separated using modified technique of Furlan *et al.*, 1980. After centrifugation in distilled water, spores were suspended in 80-90% glycerol and centrifuged at 1400 rpm for 5 min. The spores were separated by 250-350 mesh and rinsed with distilled water.

Spores were either mounted in a drop of sterile water or trypan blue in lactophenol and examined under the microscope. The Measurements were recorded both of water and stain mounts to precisely identify the spore type.

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

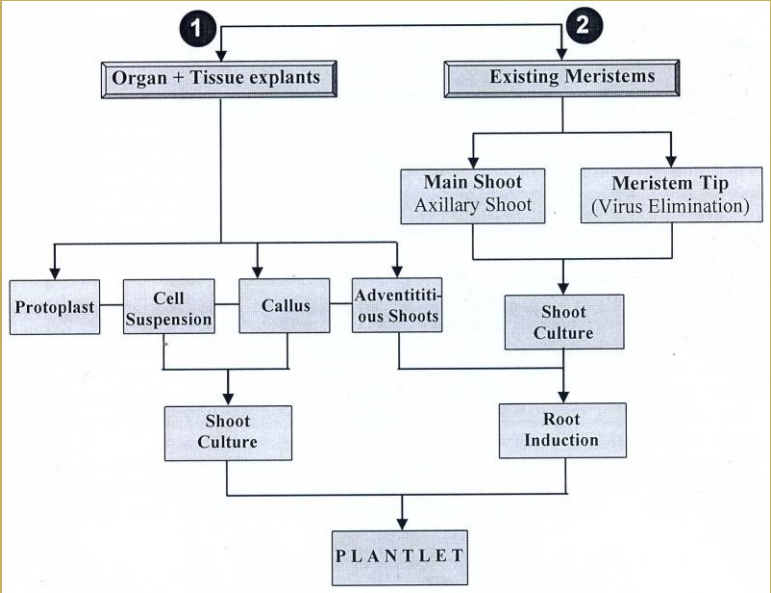


Fig. 1. Schematic diagram illustrating the pathways of regeneration in plant tissues (Modified from Short, 1990).

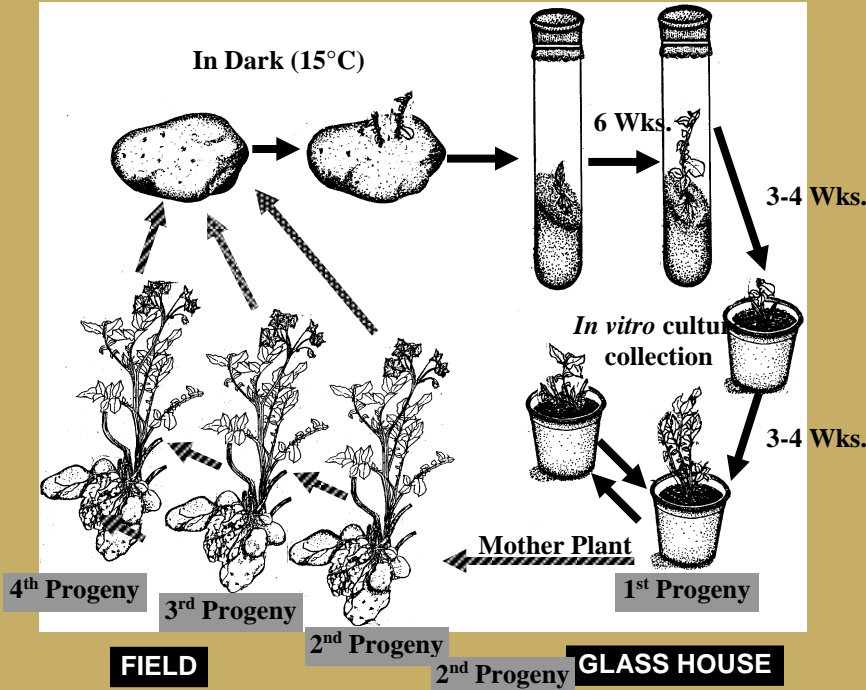


Fig. 2. Schematic diagram showing micropropagation of potato at different growth stages.

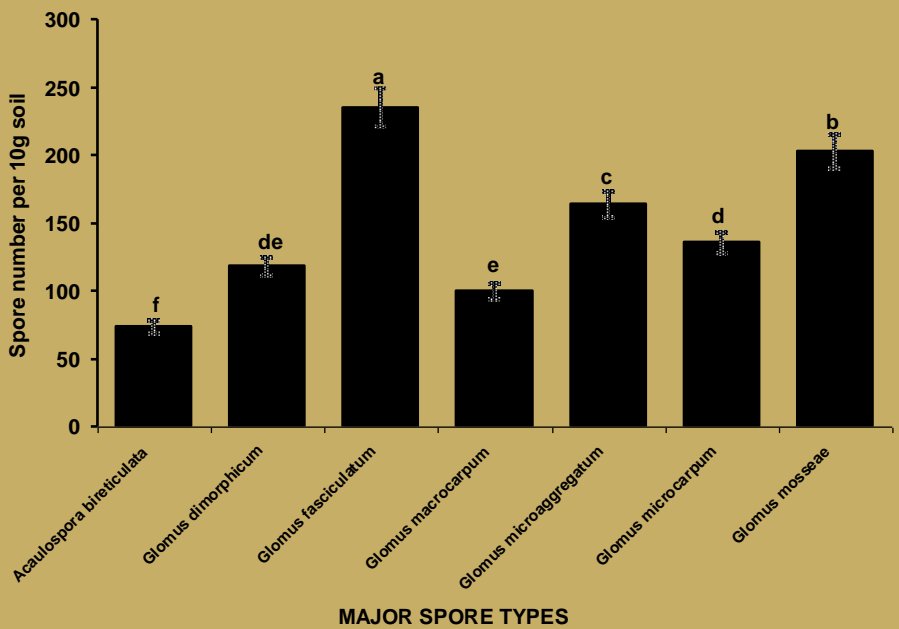


Fig. 3. Spore counts for seven major AM species in the rhizosphere of tissue cultured potato plants.

Results and Discussion

In 10g of the potato rhizosphere soil studied, the occurrence of arbuscular mycorrhizal fungi (AMF) was highly variable. Endophytes like *Glomus mosseae* and *G. fasciculatum* were highly abundant (Tables 1, 2; Fig. 3) while *Acaulospora bireticulata*, *G. dimorphicum*, *G. macrocarpum* and *G. microcarpum* were of fairly high occurrence. Spores of the species which were rarely observed were *G. albidum*, *G. claroideum*, *G. deserticola*, *G. multicaule*, unidentified species of *Glomus* one of *Scutellospora* and two totally unidentified species. The rest of the species had an intermediate frequency of occurrence. It is also evident from the results (Tables 1, 2; Fig. 3) that most (19 out of 29) of AM forming species belonged to the genus *Glomus* (Morton, 1988). This has been also pointed out by earlier workers (Nasim & Iqbal 1991a & b; Iqbal & Nasim 1991; Nasim & Bajwa, 2003; 2005; 2007) working with other crops like wheat and rice. The existence of *Glomus* as the most dominant and most common genus (65%) in the root zone of potato indicates the influence of the soil or the plant type. Moreover *Glomus* is the only mycorrhizal genus with maximum number of species.

The present study was aimed to develop an inventory of AM fungi associated with potato. This has involved a cumbersome identification process of slide preparation and matching the spore type with the descriptions (Table 1) given by standard identification manuals. Accurate identification of these fungi sometime requires them to be isolated with host plant in pot cultures, to observe developmental stages and avoid the loss of diagnostic features which occur in field collected material. In this way pure pot cultures are raised of the prominent species associated with major crop plants. The ultimate goal of this study is to eventually inoculate tissue culture raised potato plants in the field with superior strains helpful in acclimatization and to introduce other known species from other parts of the world that might be effective.

Table 1. Arbuscular mycorrhizal fungi of potato.

***Acaulospora bireticulata* Rothwell & Trappe**

Sporocarps unknown, zygospores formed singly in soil, sessile, born laterally on hyaline thin walled hyphae tapered from 2.5-7.5 μm diameter at its base to 10-30 μm diameter near its terminus, globose to sub-globose vesicles, 127-135 μm in diameter, spores bearing hypha with emergent, branched, flagellate hyphae and collapsing by maturity, spores globose 150-155 μm diameter, sub-hyaline in young, becoming light brown by maturity, spore surface ornamented with polygonal reticulum, the ridges 2x1.5-2 with sinuous, dark grayish-green sides and paler, depressed central stratum, ridges occasionally branched towards the centre of polygons or forming irregular, isolates projections at polygonal beset with round tipped 4-6 sided processes $\pm 1 \times 1 \mu\text{m}$ to give the appearance of an inverted reticulum. Spore walls of three layeres, each $\pm 1 \mu\text{m}$ thick, the outer layer dark grayish green to grayish brown, the inner layer hyaline.

***Glomus albidum* Walker & Rhodes**

Sporocarp unknown, chlamydospores under refracted hyaline when young, white to off-white at maturity, always appearing yellowish to brownish yellow by transmitted light through a compound light microscope, spore with one subtending hypha (rarely with two subtending hyphae) born singly in the soil on coenocytic hyphae. Mature spores (85) 96-168 (-198) x (85-) 95-168 (-177) μm , globose to sub-globose, occasionally ovoid or irregular, cyanophilous on cotton blue at maturity, slowly or less strongly so in youth, mature spore becoming dull orange to yellow in Melzer's reagent, young spores becoming pink to orange red. Spore walls continuous with hyphal walls, clearly double in youth, consisting of an outer hyaline wall 0.5-2 μm thick and an inner sub-equal finally laminated wall, light yellow and 0.5-2 μm thick. At maturity, the outer wall crumbling and expanding becoming as much as 8 μm thick in places and rendering the spore opaque then partly sloughing off, often becoming less than 1 μm thick and having roughened granular appearance. Subtending hyphae 2-walled, outer wall thickened at spore base (3-5) 5-15 μm wide, usually straight and simple but sometimes constricted at the spore base or expanded by thickening of outer wall to become slightly funnel shaped. Occasionally with bulging septum, 5-20 μm of the pore but usually open outer wall up to 0.7 μm thick, sloughing off at maturity to leave the inner wall (0.2-0.5 μm thick) unsupported, hypha then shriveling and collapsing often become difficult to see. Spore content of crowded oil droplets, usually becoming angular from mutual pressure to give a reticulate appearance, seemingly sealed off by collapse or the subtending hypha at maturity. Germination by germ tube penetrating the spore wall. Regrowth of subtending hypha not observed.

***G. claroideum* Schenck & Smith**

Sporocarps unknown; chlamydospores formed singly or in loose clusters in the soil and infrequently as single spores in the root. Chlamydospores globose (70-) 130(-180) μm in diameter; occasionally sub-globose to irregular; 59-126 x 72-145 μm ; spore wall (4.5-)7.6(-10.5) μm consisting of 1 or 2 walls with the outer wall laminate and

usually thicker than the inner wall; spore walls hyaline to yellow, becoming yellow-brown with age; outer spore wall smooth but frequently with soil particles or debris adhering. Spore contents globular, hyaline to light yellow, retained by a membrane on young spores and occluded by spore walls on older spores. Spore subtending hyphae 7.5-15 μm diameter at the spore attachment; walls of subtending hyphae on young spores 3.0-5.5 μm thick on older spores at the point of attachment, usually abruptly tapering below the spore; considerable branching of the subtending hyphae usually occurs 50-150 μm below the spore. Extrametrical hyphae 3-14 μm diameter with hyaline to yellow walls usually 1.5-3 μm thick. Forming typical vesicular-arbuscular mycorrhizae with plant roots.

***G. dimorphicum* Boyetchko & Tewari**

Spores dimorphic, forming singly and in loose clusters in soil; contents hyaline not reacting diagnostically with Melzer's reagent. Single spores yellow to reddish brown, globose to sub-globose, 90-300 μm . Wall of single spore of three walls organized in 2 wall groups. Sporogenous hypha of single spores straight or slightly curved. Light yellow to light brown, wall 1.5-9.0 μm thick and consisting of 1-3 walls; point of attachment occasionally possessing a septum, often occluded, flared or cylindric, 10-34 μm thick, another septum often present a little distance away from the point of attachment. Spores in clusters often arranged radiately in groups of up to 15 or more, 50-130 μm in diameter, globose to sub-globose. Walls of clustered spores of 2 distinct walls organized in 1 wall group; wall 1 laminate, 2-5 μm thick, yellow becoming reddish brown when mature; wall 2 yellow, membranous, often incipiently wrinkled in certain places, about 1 μm thick. Sporogenous hypha of clustered spores straight, forming a loose network of sparsely septate hyphae in the central part of the cluster, light yellow to brown, wall 2-6 μm thick and consisting of walls 1 and 2; point of attachment cylindric to flared, 7-12 μm thick. Sparsely septate hyphae present in the infected roots of barley, vesicles rare, arbuscules not seen.

***G. deserticola* Trappe, Bloss & Menge**

Spores borne singly or in loose fascicles in soil or within roots, globose to sub-globose (47-)54-115 x (38-)52-102 μm , shiny smooth, reddish brown, with a single, sometimes laminated wall (1.5-)2-2.5(-4) μm thick. Attached hypha 6-12 μm in diameter, cylindrical to occasionally somewhat funnel shaped, the walls, the walls thickened and reddish brown, especially thick adjacent to the spore but not occluding the hypha. Interior of the spore wall at the hyphal attachment thickened at maturity to form an inner mounded collar, which appears to be closed by a membranous septum.

***G. etunicatum* Becker & Gerd.**

Chlamydospores formed singly in soil or dead roots, globose to sub-globose 68-144 (162) in diameter, smooth or roughened from decomposition of the outer wall and adherent debris. Spore wall 4-13 μm thick, composed of an ephemeral hyaline outer wall up to 5 μm thick, and a persistent yellow to brown laminate inner wall 2-8 μm thick. Intact outer wall rarely present on mature spores, inner wall darkening and becoming laminate with age. Spores with one, rarely two hyphal attachments. Outer

wall extending down attached hypha for a short distance. Attached hypha thickened by extension of inner spore wall for up to 30 μm . Spore contents separated from attached hypha by a thin curved septum; at maturity, opening occluded by inner wall thickening.

***G. fasciculatum* (Thaxter Sensus Gerd.) Gerd. & Trappe**

Chlamydospores borne free in soil, in dead rootlets, in loose aggregations, in small compact clusters, and in sporocarps. Sporocarps up to 8 x 5 x 5 mm irregularly globose or flattened, tuberculate, grayish brown, peridium absent. Chlamydospores 35-105 μm in diameter when globose, 75-150 x 35-100 μm when sub-globose to obovate, ellipsoid, sub-lenticular, cylindrical, or irregular, smooth or seemingly roughened from adherent debris. Spore wall highly variable in thickness (3-17 μm) hyaline to light yellow or yellow brown, the thicker wall often minutely perforate with thickened inward projections. Hyphal attachments 4-15 μm in diameter, occluded at maturity. Wall of attached hyphae often thickened to 1-4 μm near the spore.

***G. halonatum* Rose & Trappe**

Chlamydospores borne singly in soil or in small, loose clusters of 3-7 spores with in a loosely vefted peridium, globose to sub-globose, 200-280 μm in diameter, light brown to brown. Spore wall 18-35 μm thick, of two layers: the outer 8-12 (-20) μm thick, hyaline, amorphous, sometimes with obscure radial striations, in youth smooth, with age becoming roughened; the inner 10-15 μm thick, brown, often prominently laminate, ornamented with crowded spines 0.5 x 0.2 μm that extends into the outer layer. Old spores sometimes with a third dark brown innermost layer \pm 5 μm thick. Attached hypha straight, extending through the outer hyaline wall wherein it is constricted to 5-6 μm in diameter. At the surface of the outer hyaline wall, subtending hypha expanded to 11-13 μm in diameter and at \pm 10 μm below the outer hyaline wall inflated to as much as 17 μm in diameter; hyphal walls near the spore totaling 5-8 μm thick, with a thick hyaline outer layer and a thin brown inner layer; hypha often with a septum \pm 30 μm below the attachment. Spore contents of oil globules of varying size.

***G. heterosporum* Smith, Schenck & Tewari**

Sporocarps light to dark brown, globose to subglobose, 242-726 x 242-641 μm , consisting of a single, ordered layer of chlamydospores originating from a central core of thick interwoven hyphae. Peridium absent. Two spore types produced. Chlamydospores produced in sporocarps, light to dark brown, obovoid to ellipsoidellipsoid, occasionally globose, 99-206 x 61-201 μm . Sporocarp aggregates occasionally formed. Spores in sporocarps maturing asynchronously by budding from a sporogenous cell. Spores with two distinct walls, inner wall laminate brown, 3-10 μm thick. Outer wall smooth, evanescent, hyaline 2-7 μm thick, frequently absent on aged spores. Hypha at point of attachment 5-31 μm wide. Spores frequently with multiple hyphal attachments in a 43:15:1 ratio of single, dual and triple attachments. Hyphal attachments frequently branched. Spore contents hyaline, non-globular, and separated from hyphal attachment by a septum. Second spore type formed singly or in unordered sporocarps in host tissues, testa or *Rhizobium* nodules. Spores with three

hyaline, globose to highly variable in shape, 31-102 x 27-68 μm in diameter. Spore wall with three hyaline walls. Inner wall membranous, up to 1 μm thick. Middle wall unit, 1-2.6 μm thick. Outer wall evanescent, less than 1 μm thick. Hypha at point of attachment 5-7 μm wide. Reaction to Melzer's reagent negative. Forming typical vesicular-arbuscular mycorrhizae.

***G. macrocarpum* Tul & Tul**

Sporocarps are fragmentary, none of the pieces are more than 5 mm in diameter. Spores are usually slightly longer than wide, sub-globose to globose, to irregular (90-) 120(-140) x (70-) 110(-130) μm . Spore wall is composed of two distinct layers, outer layer is thin (1-2 μm) and hyaline when mounted in water or glycerol, usually swelling to at least twice its original thickness in lactic acid; inner wall is yellow in section, 6-12 μm thick with series of laminations occasionally visible or rarely appearing as two distinct layers, swelling relatively little in lactic acid. Spores taper to the point of attachment of single persistent hypha. The average diameter of the hypha at this point is 16 μm . The inner wall at maturity thickens to occlude the pore of the attached hypha and the wall thickening continues into subtending hypha for upto 90 μm from the spore. Infrequently the spore seem to be closed by septum that is thinner than the normal occluding wall thickening. Spore characteristically bear a straight, long subtending hypha which may extend upto 100 μm before branching or breaking.

***G. microaggregatum* Koske, Gemma & Olexia**

Sporocarps unknown. Spores formed singly in the soil in roots, or in clusters inside dead spores of other endogonaceae: hyaline to pale yellow to brownish-yellow in transmitted light; globose to sub globose to irregular (15-) 30(-50) x (15-)30(-40) μm in diameter. Spore wall of one or two walls in one group. Attachment hypha concolorous with wall 1, straight or infundibuliform, 1.8-3(-4.5) μm wide at spore base, wall up to 1.5(-2) μm thick. Pore usually open, sometimes closed by a septum formed by wall 2 distending into the attachment hypha.

***G. microcarpum* Tul & Tul**

Chlamydospores borne free in loose aggregation, in small compact clusters in a peridium or sporocarps with a peridium, sporocarp up to 5 mm broad, irregularly globose, light brown. Peridium a thin layer of interwoven hyphae similar to those in gleba, spores interspersed but less abundantly than in gleba. In sporocarps, chlamydospores 35-45 μm in diameter, globose to sub-globose, chlamydospores free in soil 25x25 - 55x32 μm , globose, sub-globose, ellipsoid, obovoid or irregular spore wall up to 7 μm thick. Laminate, hyaline to light yellow, smooth or appearing roughened from adherent debris, opening into subtending hyphae nearly occluded in mature spores by wall thickening.

***G. monosporum* Gerd & Trappe**

Sporocarps 150-152 x 160-170 μm , globose to ellipsoid, containing mostly 1, occasionally 2, or rarely 3 chlamydospores. Peridium of branched, interwoven, thin walled hyphae, the innermost 4-10 μm in diameter, the surface hyphae 1.5-5.2 μm in diameter at maturity, developing from subtending hyphae at spore base, usually

incorporating many soil particles, degree of development variable; spores completely enclosed and obscured to enclosed but visible, partially enclosed, with only a few hyphae near base or without any enclosing hyphae. Chlamydospores of *G. monosporum* 140-330 μm in diameter, generally globose to sub-globose or rarely ellipsoid. Spore walls 4-10 μm thick, yellow to dull brown composed of thin outer wall which often flakes off and a thick inner wall; inner wall laminate, with minute, abundant to scattered echinulations that protrude into the outer wall; thickening of the inner wall extending into subtending hypha. Sub. Subtending hyphae 8-12 μm in diameter, very delicate and branched, generally strongly recurved and appressed to spore walls. Chlamydospores occasionally with 2 subtending hyphae. Spores containing oil globules or occasionally filled with hyaline, thin walled hyphae 3-6 μm in diameter.

***G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe**

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 105-310 x 110-305 μm , 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 one or occasionally with two funnel shaped bases 20-30 (-50) μm in diameter divided from subtending hyphae by curved septum. Spores were with single outer wall which was 2-7 μm thick, with a thin often barely perceptible hyaline outer membrane and a thick brownish inner layer.

***G. multicaulis* Gerd & Bakshi**

Sporocarps unknown. Chlamydospores dark brown 149-249 x 124-162 μm , ellipsoidal, broadly ellipsoidal, subglobose or occasionally triangular, with 1-4 hyphal attachments, attachments generally occurring at opposite ends of spores. Spore wall 8.6-34 μm , thickest at points of hyphal attachments, rounded projections 1.2-3.7 μm long regularly distributed over wall surface. The species is unique in normally having more than one hyphal attachment. Multiple attachments are also found in other species of *Glomus* but they are relatively infrequent. It is also distinguished by its extremely thick wall, over which rounded protuberances are evenly distributed.

***G. pallidus* Hall**

Spores formed singly or in loose clusters in soil, or in lobed or irregular epigeous sporocarps, 1-25 mm in diameter, incorporating varying amounts of soil. Sporocarps white when young turning pale yellow with age, poorly developed peridium may be present. Spores globose to sub-globose 32-78 x 28-68 μm in diameter. Wall 1-8 μm thick becoming laminated with age. Subtending hyphae 5-15 (-20) μm in diameter with pore partially occluded by the walls of the subtending hypha, pore closed by septum in some mature spores. In sporocarps new spores often formed on lateral branches of subtending hyphae of older spores. Spores sometimes appear to be intercalary due to germination in distal region. Mature glebal hyphae. Spore walls, and subtending hyphae laminated.

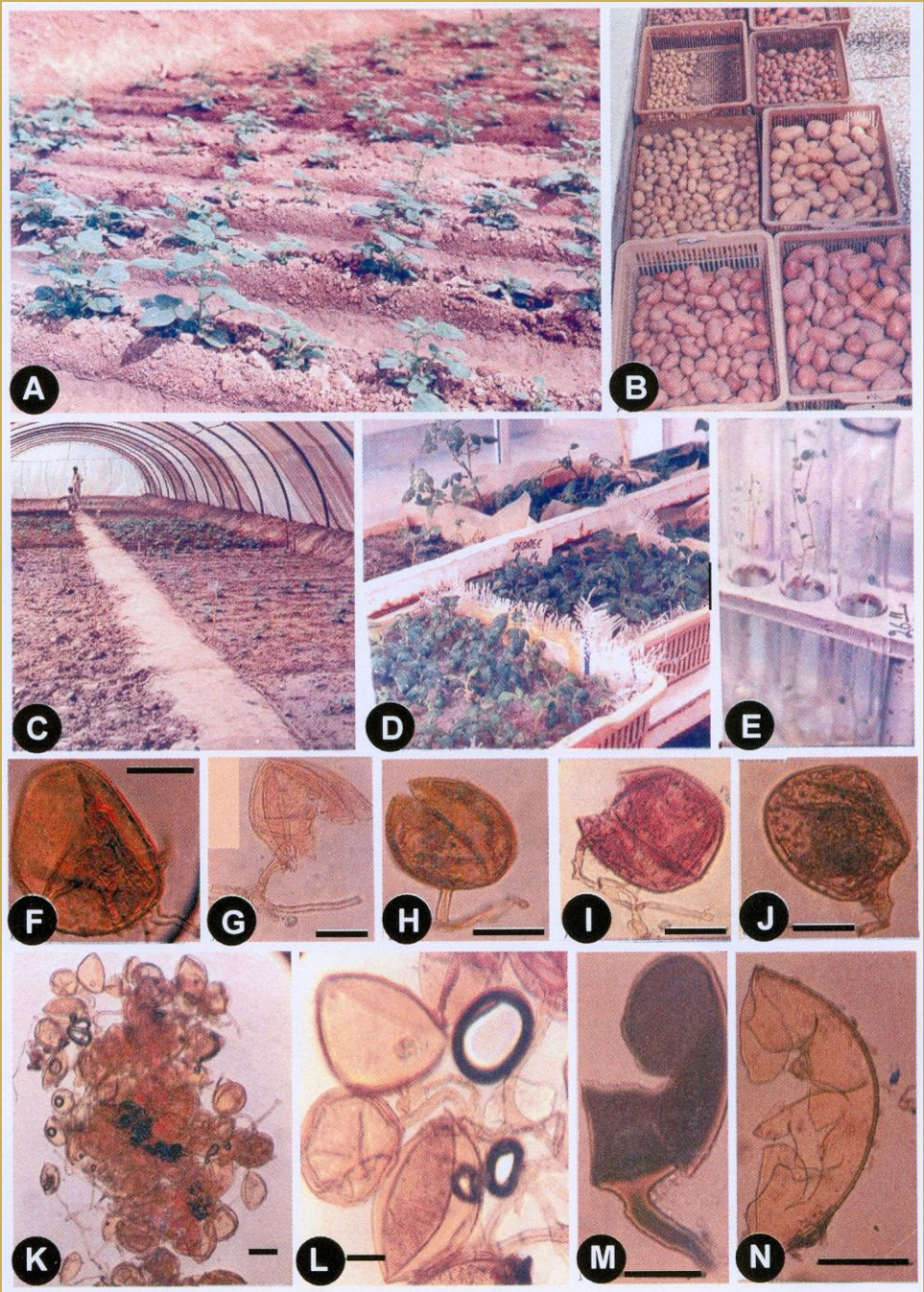


Fig. 4. A-E: Tissue culture raised potato plantlets in growth tunnels, test trays and tubes in Sahiwal area of Pakistan. F: *Glomus albidum*. G & H: *Glomus clarioideum*; I: *Glomus microcarpum*; J: *Glomus multicaule*; K & L: *Glomus mosseae*; M: *Glomus monosporum*; N: *Glomus* sp. (bar = 30 μ m).

Table 2. List of Glomaromycota spores isolated from soils of potato growing fields, Sahiwal Pakistan.

Sr. No.	Species name	% Age of spores per 100 g of soil
1.	<i>Acaulospora bireticulata</i> Rothwell & Trappe	+++
2.	<i>Acaulospora</i> sp. (Unidentified)	++
3.	<i>Gigaspora</i> sp. (Unidentified)	++
4.	<i>Glomus albidum</i> Walker & Rhodes	+
5.	<i>G. claroideum</i> Schenck & Smith	+
6.	<i>G. dimorphicum</i> Boyetchko & Tewari	+++
7.	<i>G. deserticola</i> Trappe, Bloss & Menge	+
8.	<i>G. etunicatum</i> Becker & Gerd.	++
9.	<i>G. fasciculatum</i> (Thaxter Sensus Gerd.) Gerd. & Trappe	++++*
10.	<i>G. halonatum</i> Rose & Trappe	++
11.	<i>G. heterosporum</i> Smith, Schenck & Tewari	++
12.	<i>G. macrocarpum</i> Tul & Tul	+++
13.	<i>G. microaggregatum</i> Koske, Gemma & Olexia	++++
14.	<i>G. microcarpum</i> Tul & Tul	+++
15.	<i>G. monosporum</i> Gerd & Trappe	++
16.	<i>G. mosseae</i> (Nicol. & Gerd.) Gerd. & Trappe	++++*
17.	<i>G. multicaule</i> Gerd & Bakshi	+
18.	<i>G. pallidum</i> Hall	++
19.	<i>Glomus</i> sp. I (Unidentified)	+
20.	<i>Glomus</i> sp. II (Unidentified)	+
21.	<i>Glomus</i> sp. III (Unidentified)	++
22.	<i>Glomus</i> sp. IV (Unidentified)	+
23.	<i>Sclerocystis</i> sp. (Unidentified)	+++
24.	<i>Scutellospora</i> sp. I (Unidentified)	++
25.	<i>Scutellospora</i> sp. II (Unidentified)	+
26.	Spore Type I (Unidentified)	+
27.	Spore Type II (Unidentified)	++
28.	Spore Type III (Unidentified)	++
29.	Spore Type IV (Unidentified)	+

Key: += 0-25%, += 50-75% & +++=75-100% (*Highly Abundant)

References

- Al-Karaki, G.N. 2002. Benefit, cost and phosphorus use efficiency of mycorrhizal field grown garlic at different soil phosphorus levels. *Journal of Plant Nutrition*, 25: 1175-1184.
- Augé, R.M., H.D. Toler, C.E. Sams and G. Nasim. 2008. Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza induced increases in stomatal conductance. *Mycorrhiza*, 18: 115-121
- Bajwa, R., A. Javaid and B. Haneef. 1999. EM and VAM technology in Pakistan V: Response of chickpea (*Cicer arietinum* L.) to co-inoculation with effective microorganisms (EM) and VA mycorrhiza under allelopathic stress. *Pakistan Journal of Botany*, 31: 387-396.
- Bajwa, R., J. Akhtar and A. Javaid. 2003. Role of VAM in alleviating allelopathic stress of *Parthenium hysterophorus* on maize. *Mycopath*, 1: 15-30.
- Cavagnaro, T.R., L.E. Jackson, J. Six., H. Ferris, S. Goyal, D. Asami and K.M. Scow. 2006. Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. *Plant and Soil*, 282: 209-225.
- Daniels, B.A. and H.D. Skipper. 1982. Methods of recovery and quantitative estimation of propagules from soil. In: *Methods of Mycorrhizal Research*. (Ed.): N.C. Schenck. Published by American Phytopathological Society, St. Paul, Minnesota.
- Furlan, V., H. Bartschi, H and J.A. Fortin. 1980. Media for density Gradient Extraction of Endomycorrhizal spores. *Trans. Br. Mycol. Soc.*, 75: 336-338.
- Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal 'Endogone' extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, 84: 679-684.
- Iqbal, S.H and G. Nasim. 1986. Vesicular arbuscular mycorrhiza in roots and other underground portion of *Curcuma longa*. *Biologia*, 32(1): 223-228.
- Iqbal, S.H. and G. Nasim. 1991. Are under ground non-root portions of tropical plants vesicular arbuscular mycorrhizal ? *Transactions of the Mycological Society of Japan*, 32: 467-476.
- Javaid, A. 2007. Allelopathic interactions in mycorrhizal associations. *Allelopathy Journal*, 20(1): 29-42.
- Javaid, A., F.Y. Hafeez and S.H. Iqbal. 1993. Interaction between vesicular arbuscular (VA) mycorrhiza and *Rhizobium* and their effect of biomass, nodulation and nitrogen fixation in *Vigna radiata* (L.) Wilczek. *Science International (Lahore)*, 5: 395-396.
- Javaid, A., S.H. Iqbal and F.Y. Hafeez. 1994. Effect of different strains of *Bradyrhizobium* and two types of vesicular arbuscular mycorrhizae (VAM) on biomass and nitrogen fixation in *Vigna radiata* (L.) Wilczek var. NM 20-21. *Science International (Lahore)*, 6: 265-267.
- Johnson, D., J. Leake and D.J. Read. 2006. Role of arbuscular mycorrhizal fungi in carbon and nutrient cycling in grassland. In: *Fungi in Biogeochemical cycles*, (Ed.): G.M. Gadd. Published by Cambridge University Press. © British Mycological Society.
- Khaosaad, T., J.M. Garcia-Garrido, S. Steinkellner and H. Vierheilig. 2007. Take-all disease is systemically reduced in roots of mycorrhizal barley plants. *Soil Biology and Biochemistry*, 39: 727-734.
- Krishna, K.R. 2005. *Mycorrhizas: A Molecular Analysis*, Science Publishers, Inc. Enfield, New Hampshire, USA.
- Morton, J.B. 1988. Taxonomy of VA mycorrhizal fungi: Classification, nomenclature and identification. *Mycotaxon*, 32: 267-324.
- Nasim, G and R. Bajwa. 2005. Glomalean spores associated with major cereals I-Wheat *Caderno Pesquisa Serie Biologia*, 17(1): 137-154.
- Nasim, G and S.H. Iqbal. 1991b. Fate of Endogonaceous spores in soil. *Transactions of the Mycological society of Japan*, 32: 517-522.
- Nasim, G. and R. Bajwa. 2003. Endogonaceous spore flora of Pakistan. IX. Frequency of occurrence of VAM fungi in wheat fields around Punjab University Campus Area. *Mycopath*, 1(1): 67-80.
- Nasim, G. and R. Bajwa. 2007. Seasonal dynamics and relative abundance of AM fungi in rhizosphere of rice (*Oryza sativa* L. cv. Basmati Supper). *Mycopath*, 5(1): 53-64.

- Nasim, G. and S.H. Iqbal. 1991a. Species of *Glomus* associated with non-root portions of some rhizomatous plants and characteristics of their mycorrhizae. *Transactions of the Mycological Society of Japan*, 32: 541-545.
- Phillips, J.M. and D.S. Hayman. 1970. Improved procedure for clearing roots and staining parasitic and VA mycorrhizal fungi for rapid assessment of infection. *Transactions of British Mycological Society*, 5: 158-161.
- Ruiz-Lozano, J.M. 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. *Mycorrhiza*, 13: 309-317.
- Schenck, N.C and Y. Perez. 1987. *Manual for identification of VA mycorrhizal fungi*. Field Hall, University of Florida, Gainesville, Florida.
- Schliemann, W., C. Ammer and D. Strack. 2008. Metabolite profiling of mycorrhizal roots of *Medicago truncatula*. *Phytochemistry*, 69: 112-146.
- Schüßler, A., D. Schwarzott and C. Walker. 2001. A new fungal phylum, the Glomaromycota: phylogeny and evolution. *Mycol Research*, 105: 1403-1421.
- Short, K.C. 1990. Application of *In vitro* techniques for the production and the improvement of Horticultural Plants. In: *Impact of Biotechnology in Agriculture*. 15-27. (Eds.): R.S. Sangwan and B. S. Sangwan, Kluwer Academic. Printed in the Netherlands.
- Short, K.C. 1991. The physiology of cultured plantlets and methods for facilitating their transfer to field conditions. In: *Conservation of Plant Genetic Resources through In Vitro methods*. 65-73.
- Takeda, N., C. Kistner, S. Kosuta, T. Winzer, A. Pitzschke, M. Groth, M. Sato, T. Kaneko, S. Tabata and M. Parniske. 2007. Proteases in plant root symbiosis. *Phytochemistry*, 68: 111-121.
- Trappe, J.M. 1992. Synoptic key to the genera and species of Zygomycetous mycorrhizal fungi. *Phytopathology*, 72(8): 1102-1108.
- Wu, O.S. and R.X. Xia. 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology*, 163: 417-425.
- Wu, O.S., R.X. Xia and Y.N. Zou. 2008. Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *European Journal of Soil Biology*, 44: 122-128.

(Received for publication 14 September 2009)