REVIEW OF LITERATURE
SECTION I - HISTORY OF ARBUSCULAR MYCORRHIZAL FUNGI:

Arbuscular Mycorrhizal (AM) fungi have been described as early as 1842 (Nageli, 1842) but most of Nageli’s drawings only remotely resembled the AM fungi. Trappe and Berch (1985) and Rayner (1926, 1927) cited other earlier observations of the symbiosis during the period 1875-1895. Extensive surveys of host plants and anatomical descriptions on AM are given by Schlicht (1889), Dangeard (1896), Janse (1897), Petri (1903), Gallaud (1905), Peyronel (1924) and Lohman (1927). As early as 1889, Schlicht had already observed the basic anatomical relationships between the host and fungal tissues. Janse (1897), called the intramatrical spores “vesicles” and determined that other structures, named “arbuscules” by Gallaud (1905), were located in the inner cortex. Gallaud (1905) made a very accurate observation of the arbuscule and concluded that it is surrounded by a host membrane, which was later confirmed by Cox and Sanders (1974) using transmission electron microscopy. Gallaud (1905) also noted that partial digestion of the arbuscule resulted in a structure called the “sporangiole” by Janse (1897). This observation was confirmed by electron microscopy (Cox and Sanders, 1974). Gallaud (1905) further distinguished between Arum and Paris types of arbuscules (Smith and Smith, 1997). Jones (1924) described the term “apressorium”. Link (1809) established the genus *Endogone*. Tulasne and Tulasne (1844) were the first to describe the genus *Glomus*, known only from spore clusters found in soil. Dangeard (1896) was the first to describe an arbuscular mycorrhiza, which happened to have formed from poplar roots.
Frank (1885) gave the name “mycorhiza” to the peculiar association between tree roots and ectomycorrhizal fungi. A thorough discussion of the derivation of the word “Mycorrhiza”, including the incorporation of the second ‘r’ is given Kelly (1931, 1950). The name for the AM symbiosis has changed throughout the years. The symbiosis was once frequently called “Phycomycetous endomycorrhiza” to distinguish it from the endomycorrhizal symbiosis formed between members of the Ericaceae or Orchidaceae and higher fungi. The recognition that not all fungi formed vesicles led to the proposal that this symbiosis should be renamed Arbuscular mycorrhiza (Koide and Mosse, 2004).

The evolution of mycorrhizal symbiosis:

Paleobiology is a field dealing with the biological and ecological functions that can be deduced from fossils. Both paleobiological and molecular evidence indicate that AM is an ancient symbiosis that originated 460 million years ago. Arbuscular mycorrhizal symbiosis is ubiquitous among land plants, which suggest that mycorrhizae were probably present in the early ancestors of land plants. This positive association with plants may have facilitated the development of land plants (Simon et al., 1993).

The Rhynie chert (siliceous rock of chalcedonic or opaline silica occurring in limestone) of the lower Devonian has yielded fossils of the earliest land plants in which AM fungi have been observed. The fossilized plants containing mycorrhizal
fungi were preserved in silica. They are prepared for observation by cementing pieces of the rock to microscope slides and then grinding the rock with carbide powder to a thickness of 50-150μm (Remy et al., 1994).

The Early Devonian saw the development of terrestrial flora. Plants of the Rhynie chert from the lower Devonian (400 mya) were found to contain structures resembling vesicles and spores of present Glomus species. Colonized fossil roots have been observed in Aglaophyton major and Rhynia, which are ancient plants possessing characteristics of vascular plants and bryophytes with primitive protostelic rhizomes (Remy et al., 1994). Intraradical mycelium was observed in root intracellular spaces and arbuscules were observed in the layer of thin cell walls similar to palisade parenchyma. The fossil arbuscules appear very similar to those existing AM fungi (Remy et al., 1994). The cells containing arbuscules have thickened walls, which were also observed in colonized cells. Kar et al. (2005) have recently uncovered mycorrhizae from the Miocene, which exhibit a vesicular morphology closely resembling that of the present Glomerales. The need for further evolution may have been lost due to the readily available food source provided by the plant host (Kar et al., 2005). The nature of the relationship between plants and the ancestors of AM fungi are characterized as follows:

- Mycorrhizal symbiosis may have evolved from a parasitic interaction which developed into a mutually beneficial relationship.
An alternate hypothesis is that mycorrhizal fungi developed from saprophytic fungi that became endosymbiotic (Remy et al., 1994).

There is some fossil evidence that the parasitic fungi did not kill the host cells immediately upon invasion although a response to the invasion was observed in the host cell. This response may have evolved into the chemical signaling processes required for symbiosis (Remy et al., 1994). In both cases, the plant fungal interaction is thought to have evolved from a relationship in which fungi was taking nutrients from the plant into a symbiotic relationship where the plants and fungi exchanged nutrients.

**The symbionts:**

Arbuscular mycorrhizae are thought to be ecologically important to most vascular plants. Mycorrhizal fungi are found in most of the herbaceous plants and also in tree species (Harley and Smith, 1983). Plants classified as bryophytes, pteridophytes, gymnosperms and angiosperms have been found to form mycorrhizas. Most plants are capable of forming mycorrhizae with numerous species of AM fungi. The persistence of the relationship indicates that mycorrhizae confer some evolutionary advantage to plants in the form of nutrient uptake. Plants with roots that present little branching or lack of fine root hairs, relatively inefficient at seeking out P may receive maximum benefit from mycorrhizal symbiosis (Harley and Smith, 1983).
SECTION II- CLASSIFICATION:

Arbuscular mycorrhizal fungi comprise a monophyletic group of soil fungi, recently reclassified from the polyphyletic phylum Zygomycota to a newly proposed phylum Glomeromycota (Schüster et al., 2001). This phylum was proposed after analysis of a large data set of 18S rRNA gene sequences of all known groups of fungi. To date, more than 190 AM fungal species have been described. They are classified in four orders encompassing 10 families and 14 genera (Table 1). *Glomus* is the largest genus containing 54.8% of all described species. In phylogenetic tree based on rDNA, Glomeromycota are sister group to Asco- and Basidio-mycota. The original taxonomy of the AM fungi was based on the morphology of the large soil borne spores, which were found near colonized plant host roots. Distinguishing mycorrhizal spore characteristics used in classification include wall morphologies, size, shape, colour, hyphal attachment and reaction to staining compounds (Wright, 2005).

The ancient phylogenetic origin of Glomales is confirmed by fossil findings, with symbiotic structures within fossil roots from Devonian (Remy et al., 1994; Taylor et al., 1995) and fossilized glomalean spores from the Ordovician, about 460 million years ago (Redecker et al., 2000). Since molecular phylogenetic methods have been used to elucidate the phylogenetic relationships among these fungi, their classification has been in a rapid transition. Molecular studies have also revealed a large number of putative new species suggesting that the 150 morphologically-
Table 1: The classification of AM fungi by Schüßler et al. (2001) with emendations of Oehl and Sieverding (2004), Sieverding and Oehl (2006), Spain et al. (2006), and Walker and Schüßler (2004), Walker et al. (2007a, b) and Palenzuela et al. (2008).

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<th>PHYLUM</th>
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<td>CLASS</td>
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<td>GENUS</td>
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<td>1. Archaeosporales</td>
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<td>2. Diversisporales</td>
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<td>Entrophospora</td>
<td>Ames &amp; Schneid. emend. Oehl &amp; Sieverd</td>
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**GIGASPORACEAE** Morton & Benny

**GIGASPORA** Gerd. & Trappe emend. Walker & Sanders

**SCUTELLOSPORA** Walker & Sanders

**PACISPORACEAE** Walker, Blaszk., Schüßler & Schwarzott

**PACISPORA** Oehl & Sieverd.

3. **GLOMERALES** Morton & Benny

**GLOMERACEAE** Piroz. & Dalpe

**GLOMUS** Tul. & Tul

4. **PARAGLOMERALES** Walker & Schüßler

**PARAGLOMACEAE** Morton & Redecker

**PARAGLOMUS** Morton & Redecker
defined species may vastly underestimate species diversity (Vandenkerckhuyse et al., 2002). Traditionally, glomeromycotan taxonomy is mainly based on the morphology of spores. The way the spore is formed on the hypha (mode of spore formation) has been important to circumscribe genera, families, and spore wall structure to distinguish species (Walker, 1983; Morton, 1988).

Prior to 1974, most AM fungi were in the genus Endogone. However, Gerdemann and Trappe (1974) removed AM fungi from Endogone and placed them in four separate genera viz., Glomus, Sclerocystis, Acaulospora and Gigaspora. Unlike the putatively asexual members of the Glomeromycota, Endogone species reproduce sexually via zygospores, indicating their phylogenetic link with the phylum Zygomycota. Phylogenetic analysis of the nuclear small subunit ribosomal RNA strongly suggest that Endogone (Endogonales) and the Glomeromycota do not form a clade (Gehrig et al., 1996).

The order Glomales was erected by Morton and Benny (1990) for AM fungal classification with two more genera, Scutellospora and Entrophospora and three families (Glomaceae, Gigasporaceae and Acaulosporaceae). These families were characterized by the mode of spore formation and were initially supported by molecular data (Simon et al., 1993).
Spores of *Glomus* and *Acaulospora* types were reported to be produced by several distinct, deeply divergent lineages (Redecker et al., 2000a) subsequently, described as two new genera *Archaeospora* and *Paraglomus* (Morton and Redecker, 2001) and placed in separate families. Because some species in *Archaeospora* were dimorphic, members of this genus were classified originally in separate families (Morton et al., 1997).

Molecular phylogenetic analysis has also shown that the species which form complex sporocarps formerly placed in the genus *Sclerocystis* are actually phylogenetically nested within well characterized *Glomus* species with simple spores (Redecker et al., 2000b). The genus *Pacispora* comprising of some former *Glomus* species was erected by Oehl and Sieverding (2004). The spores of *Pacispora* have characteristics intermediate between *Glomus* and Gigasporaceae. Another emerging genus split off from *Glomus* is *Diversispora* (Morton and Benny, 1990). Only one *Glomus* species has been formally renamed so far, mainly based on ribosomal small subunit signatures (Walker and Schubler, 2004). The new classification includes the Geosiphonaceae; order Archaeosporales, which presently contain one fungal species that forms endosymbiotic association with the cyanobacterium *Nostoc punctiforme* and produce spores typical to AM fungi (Schubler, 2002).

The Glomeromycota are very old group with an estimated origin of at least 600 to 620 million years ago. Spores and hyphae of Glomalean fungi were discovered
in 460 million year old rocks from the Ordovician thus making them the oldest recognized fungal fossils to date (Redecker et al., 2000b).

**Phylogenetic Relationships:**

In the recent classification, the phylogeny erected is based entirely on analyses of the small subunit RNA gene. rDNA phylogenies have shown that the genus *Glomus* is several times polyphyletic (Redecker et al., 2000b; Schwarzott et al., 2001). Species forming *Glomus*-like spores can be found in six different lineages within the Glomeromycota. *Paraglomus* appears to be the earliest-diverging glomeromycotan lineage in rDNA phylogenies, although sometimes receiving relatively weak bootstrap support. The separation of *Pacispora* and the *Diversispora* clade from other "*Glomus*" lineages is well supported by rDNA data. *Glomus* groups A and B are exemplified by the well-known species of *Glomus mosseae* and *G. claroideum* respectively. The two groups are genetically relatively distant but still form a monophyletic group in rDNA phylogenetic trees (Schwarzott et al., 2001).

The formation of a "sporiferous saccule" was once thought to be characteristic of Acaulosporaceae (*Acaulospora* and *Entrophospora*), but now is known to occur in at least one additional lineage, namely *Archaeospora*. The Gigasporaceae (*Scutellospora* and *Gigaspora*) are distinguished by the formation of their spores on a "bulbous suspensor" and are well supported by molecular data. Gigasporaceae and Acaulosporaceae form a clade in most rDNA phylogenies, which is in conflict with
previous morphology-based analyses that placed *Glomus* and Acaulosporaceae together (Morton and Benny, 1990). The fungi of the Glomeromycota have coenocytic to sparsely septate mycelia. They reproduce asexually through blastic development of the hyphal tips and form symbiotic relationships with photoautotrophs.

Unusual polymorphism of ribosomal RNA in individual spores has led to the concept of internuclear variation in single spores, defining AM fungi as heterokaryotic organisms (Trouvelot et al., 1999; Kuhn et al., 2001). Heterokaryosis has been assumed to be of importance to ecology and application of AM fungi. This concept however has recently been challenged by experiments suggesting that single spores contain uniform population of nuclei characterized by intranuclear polymorphism (Pawlowska and Taylor, 2004).

**Taxonomy:**

Peyronel (1923) discovered that the regular occurrence of associations of spores and sporocarps of the Endogonaceae with AM fungi of plants and suggested fungi to be the originators of the mycorrhizae. Valuable data on the biology of fungi of the family Endogonaceae has been obtained from studies using pot cultures. The mode of germination of spores of these fungi, their life cycles, subcellular spore structures and the manner of colonization of roots has been recognized (Mosse, 1959,
Mosse and Bowen (1968) prepared the first key for the recognition of the types of isolated endogonaceous spores.

Gerdemann and Trappe (1974) revised the family Endogonaceae in the order Mucorales, where 44 species belonging to seven genera were characterized. Among them, many taxa were redefined, and two genera (*Acaulospora, Gigaspora*) and 12 species were described as new. The genus *Endogone* contained 11 species with zygospores arranged in sporocarps. Tulasne and Tulasne (1845) erected the genus *Glomus* with 19 species with two varieties of *Gl. macrocarpus* Tul. & Tul., i.e., *Gl. macrocarpus* var. *macrocarpus* and *Gl. macrocarpus* var. *geosporus*, and also the genus *Sclerocystis* with four taxa containing species forming chlamydospores blastically at hyphal tips. In contrast to sporocarpic *Sclerocystis* spp., the chlamydospores of members of the genus *Glomus* have been considered to occur mainly in loose aggregates or singly in the soil, although the genus also included species forming compact sporocarps with or without a peridium. The distinctive property of genus *Sclerocystis* was the production of chlamydospores arranged in a single layer around a central plexus.

The genera *Acaulospora* and *Gigaspora* have been defined by Gerdemann and Trappe (1974) as forming azygospores singly in the soil, although no parthenogenetical process of spore development was observed. Species of *Acaulospora* produced spores laterally on the neck of a sporiferous saccule and
species of the genus *Gigaspora* formed spores terminally at the tip of a bulbous sporogenous cell.

Ames and Schneider (1979) erected a new genus in Endogonaceae, *Entrophospora* with *E. infrequens*, a species earlier existing in genus *Glomus*, as *Gl. infrequens*. Spores of *E. infrequens* were formed inside the neck of a sporiferous saccule.

Walker and Sanders (1986) separated the genus *Gigaspora*, containing species with spores lacking an inner wall having no physical contact with their main structural wall, from genus *Scutellospora* with fungi forming spores having at least one inner wall.

Morton and Benny (1990) located soil-borne fungi forming arbuscules in roots of terrestrial plants in new order, Glomales consisting of two suborders, Glomineae and Gigasporineae. The former suborder consisted of the type family Glomaceae with genera *Glomus* and *Sclerocystis* and Acaulosporaceae comprising of the genera *Acaulospora* and *Entrophospora*. The latter suborder was proposed to include the Gigasporaceae with the genera *Gigaspora* and *Scutellospora*. Apart from differences in the mode of formation of spores and their sub cellular structure, the taxa of the suborder Glomineae distinguished the ability to form vesicles that did not occur in mycorrhizae of members of the suborder Gigasporineae.
Almeida and Schenck (1990) concluded that except for *Sclerocystis coremioides* a continuum of morphological properties exists between sporocarpic *Glomus* species and the other members of the genus *Sclerocystis*. As a result, the five-species genus *Sclerocystis* was reduced to single-species.

Redecker *et al.* (2000) utilizing both morphological and molecular data transferred *S. coremioides* to genus *Glomus* and thereby eliminated the genus *Sclerocystis* from Kingdom Fungi. Morton and Redecker (2001) based on data from molecular, morphological and biochemical investigations, erected two new families in the order Glomales, i.e., Archaeosporaceae and Paraglomaceae. Each of these families was phylogenetically distant from other glomalean families, despite similarities in mycorrhizal morphology. The family Archaeosporaceae contained one genus, *Archaeospora* with three species forming typical *Acaulospora*-like spores from the neck of a sporiferous saccule. Two of these species, *Ar. gerdemannii* and *Ar. leptoticha* were considered to dimorphic, forming *Glomus*-like spores. The genus *Paraglomus* in the family Paraglomaceae consisted of two species producing spores indistinguishable from those of *Glomus* species.

The fungi of the order Archaeosporales form endocytosymbioses with photoautotrophic prokaryotes [*Geosiphon pyriformis* (Kütz.) Wettstein emend. Schüßler] produce mycorrhizae with arbuscules with or without vesicles. Their spores are colourless and do not react in Melzer's reagent. Glomoid spores (identical to those
of fungi of the genus *Glomus*) form singly or in clusters on or under the soil surface. Acaulosporoid spores (similar to those of members of the genus *Acaulospora*) develop singly in the soil. They differ from other AM fungi by the possession of the rRNA SSU gene signature YCTATCYKYCTGGTGAKRCG, corresponding to homologous position 691 of *Saccharomyces cerevisiae* SSU rRNA sequence J01353, with the nucleotides being specific for the taxon. The order Archaeosporales contains two families, Archaeosporaceae with the genera *Appendicispora*, *Archaeospora* and *Intraspora* and Geosiphonaceae with the genus *Geosiphon*.

Members of the order Diversisporales form mycorrhizae with arbuscules, frequently lacking vesicles, with or without auxiliary cells. Spores develop either inside (entrophosphoroioid spores of the genera *Entrophospora* and *Kuklospora*) or laterally on the neck of a sporiferous saccule (acaulosporoid spores of the genus *Acaulospora*), from a bulbous base on the sporiferous hypha (gigasporoid spores of the genera *Gigaspora* and *Scutellospora*), or blastically at the tip of a sporogenous hypha (glomoid spores of the genera *Diversispora* and *Pacispora*). They differ from other AM fungi by the possession of the rRNA SSU gene sequence signature YVRRYW/1-5/NGYYGB, corresponding to homologous position 658 of *S. cerevisiae* SSU rRNA sequence J01353 SSU rRNA, GTYARDYHMHY/2-4/GRADRKKYGYWCRAC, corresponding to homologous position of *S. cerevisiae* SSU rRNA sequence position 1346 of *S. cerevisiae* SSU rRNA sequence J01353, TTATCGGTTRAATC, corresponding to homologous position 650 of *S. cerevisiae*
rRNA SSU sequence J01353, and ACTGAGTTMATYT, corresponding to homologous position 1481 of S. cerevisiae rRNA SSU sequence J01353 with the nucleotides being specific for the taxon. The order Diversisporales is represented by five families, Diversisporaceae with the genus Diversispora, Acaulosporaceae with the genera Acaulospora and Kuklospora, Entrophosporaceae with the genus Entrophospora, Gigasporaceae with the genera Gigaspora and Scutellospora and Pacisporaceae with the genus Pacispora.

Fungi of the order Glomerales usually are hypogeous, rarely epigeous. They produce mycorrhizae with arbuscules, vesicles and spores. Spores form either blastically at the tip of a sporogenous hypha or intercalary inside them. Spores occur singly, in clusters or sporocarps having a peridium. They differ from other AM fungi by the possession of the rRNA SSU gene sequence signature YTRRY/2-5/RYYARGTYGNCARCTTTTAGAGGGACTATCGGTGTYTAACCGRTGG, corresponding to homologous position 1353 of S. cerevisiae SSU rRNA sequence J01353, with the nucleotides being specific for the taxon. The order Glomerales includes one genus, Glomus.

Species of the order Paraglomerales form arbuscular mycorrhizae, rarely with vesicles. Spores are glomoid and colourless. The fungi differ from other AM fungi by the possession of rRNA SSU gene sequence signature GCGAAGCGTCATGGCCTTAACCGGCATCTTTAGAGGGACTATCGGTGTYTAACCGRTGG, corresponding to homologous
position 703 of S. cerevisiae SSU rRNA sequence J01353, with the nucleotides being specific for the taxon. The order Paraglomerales is represented by one family Paraglomeraceae containing one genus, Paraglomus.

**Genera of Arbuscular Mycorrhizal fungi:**

Arbuscular mycorrhizal fungi are placed in four orders, viz., Archaeosporales, Diversisporales, Glomerales and Paraglomerales belonging to the class Glomeromycetes of the phylum Glomeromycota (Schüßler et al., 2001) under 14 genera viz., *Acaulospora, Archaeospora, Ambispora, Diversispora, Entrophospora, Gigaspora, Glomus, Intraspora, Kuklospora, Pacispora, Paraglomus, Scutellospora, Otospora* and *Geosiphon*.

The different genera of AM fungi are described below:


**Etyomolgy:** Greek, *a-* (without), *caulos* (stem), and *spora* (spore) - referring to the sessile spores.

Spores of fungi of the genus *Acaulospora* develop laterally from the neck of a sporiferous saccule (Morton and Benny, 1990; Morton 2000). The spores are sessile, i.e. no pedicel (a short branch of the sporiferous saccule neck) is formed. The wall of the most juvenile spores consists of only one layer continuous with the wall of a sporiferous saccule hypha. Spores produced singly in soil, generally globose to
subglobose with oily contents. Spore composed of two distinct, separable wall groups; outer wall is continuous laminated; variously ornamented, inner wall composed of one or more walls that are membranous, hyaline, laminated and ornamented. Spore walls are continuous except for a small-occluded pore. Spores of the genus *Acaulospora* germinate by germ tubes emerging from a plate-like germination orb formed by centrifugally rolled hyphae (Blaszkowski, 1994). The germ tubes penetrate through the spore wall. The mycorrhizae of *Acaulospora* species consist of (1) arbuscules with cylindrical or slightly flared trunks (2) irregular and knobby vesicles, and (3) straight and coiled intraradical hyphae with coils mostly concentrated at entry points (Morton, 2000).


**Etymology:** Greek, *giga* (giant) and *spora* (spore). Referring to the exceptionally large spores typically produced by the members of the genus.

Azgospores produced singly in soil, generally globose to subglobose, with oily contents, usually with a narrow hypha extending from the suspensor cell to the pore. Spores of *Gigaspora* develop blastically from a bulbous sporogenous cell formed at the end of a fertile hypha connected with mycorrhizal roots (Bentivenga and Morton, 1995; Walker and Sanders, 1986). The wall of the most juvenile, expanding spores consists of two layers of equal thickness. The inner layer thickens due to the synthesis of new sub layers (laminae). At the end of ontogeny, a warty or
knobby one-layered germination wall is formed, from which germ tubes arise. This wall tightly adheres to the inner surface of the laminate spore wall layer. The outermost spore wall layer of all the *Gigaspora* species is smooth. Apart from spores, *Gigaspora* species also form clusters of auxiliary cells. They are echinulate with spines. The mycorrhizae of *Gigaspora* species consist of only arbuscules and hyphae staining darkly in trypan blue; no vesicles are produced (Bentivenga and Morton, 1995). Arbuscules generally form fine branches directly from a swollen basal hypha. Intraradical hyphae are straight to coiled and vary in diameter because of the presence of knob-like projections and inflated areas.


**Etymology:** Latin *scutellum*- small shield and *spora*, spore referring to the production of germination shield in spores of members of the genus.

Spores produced singly in soil variable in shape, usually globose or subglobose often ovoid, obovoid, pyriform or irregular borne on a bulbous sporogenous cell formed at the end of a fertile hypha connected with mycorrhizal roots (Walker and Sanders, 1986) usually with a narrow hypha extending from one or more peg-like projections towards the spore. Spore wall structure consists of two wall groups of equal thickness. The inner layer thickens due to addition of new sub layers (laminae). The formation of spore wall ends the differentiation of a third thin, flexible layer, which is tightly adherent to the laminate layer. Germination by means of one or...
more germ tubes produced from the spore base through the germination shield formed upon or within a flexible inner wall. The mycorrhizae of *Scutellospora* species consist of only arbuscules and hyphae staining darkly in trypan blue; no vesicles are produced (Morton, 2000). Arbuscules develop from swollen basal hyphae. Intraradical hyphae are straight or coiled and vary in diameter because of the presence of knob-like projections and inflated areas. Thin-walled, knobby or broadly papillate auxiliary cells borne in soil on straight or coiled hyphae, formed singly or in clusters.


**Etymology:** Latin, glomus (a ball of yarn), possibly in reference to sometimes rounded and cottony appearance of the species.

Spores of *Glomus* species develop blastically at the end of sporogenous hyphae, although intercalary spore formation has also been reported (Declerck *et al.*, 2000). In most species, the sporogenous hyphae develop from extraradical hyphae of mycorrhizal roots. The surface of spores of *Glomus* species may be smooth (in most species) or ornamented. Some species produce spores enveloped in a hyphal mantle 'Gleba' consisting of interwoven 'Peridium'. The wall layers of a subtending hypha are continuous with spore wall layers. At the end of spore development, the lumen of the subtending hypha usually becomes closed by either (1) a curved septum continuous with the innermost lamina of the laminate spore wall layer (2) an invaginated flexible innermost layer (3) an amorphous plug and (4) thickening
subtending hyphal wall. Spores of the genus *Glomus* germinate by emergence of the germ tube through the lumen of the subtending hypha (most species) or the spore wall. Most species of the genus *Glomus* produce spores singly in the soil. Other taxa form more or less compact spore aggregates consisting of spores and a peridium. The mycorrhizae of *Glomus* species consist of arbuscules, vesicles (not always formed), and intra- and extra-radical hyphae. Arbuscules have cylindrical or slightly flared trunks with branches progressively tapering in width toward tips. Vesicles usually are thin-walled and ellipsoid. Intraradical hyphae usually spread along roots and frequently form Y-shaped branches, H-shaped connections and coils mainly occur at entry points.


Spores occur singly in the soil or in roots. The spores develop inside the neck of a sporiferous saccule at some distance from the saccule. The sporiferous saccule originates terminally or intercalary in extra- and intra-radical hyphae. The spores are globose to subglobose and frequently pyriform. Their sub cellular structure consists of two walls, a spore wall and an inner germination wall. The spore wall is composed of two layers, of which the outer layer sloughs with age and is continuous with the wall of the neck of the sporiferous saccule. The inner layer of this wall is persistent, semi flexible and closes two opposite pores of spores. The inner germination wall is semi-flexible and laminate. The mycorrhizae comprise of arbuscules, vesicles as well
as intra- and extra-radical hyphae. Vesicles form rarely and all the mycorrhizal structures stain faintly in trypan blue.


Spores develop inside the neck of a sporiferous saccule at some distance from this saccule and originate from the neck and saccule contents. The sporiferous saccule originate terminally or intercalary inside mycorrhizal extraradical hyphae by their swelling and are globose to subglobose. The sub cellular structure consists of a 3-layered, coloured spore wall and two inner colourless germination walls. The outermost spore wall layer is colourless, and is continuous with the wall of the sporiferous saccule neck. The second structural layer of this wall consists of coloured, tightly adherent, thin sub layers (laminae). This layer occasionally develops towards the saccule, forming a stalk supporting the wall of the sporiferous saccule neck. The first inner germination wall consists of two adherent flexible to semi-flexible layers. The second germination wall is composed of three layers, of which the outermost one is ornamented with small granules. The spores forms typical mycorrhizae intensively stained in trypan blue.

Spores of fungi of the genus *Pacispora* develop blastically at the end of cylindrical sporogenous hyphae (subtending hyphae) continuous with extraradical hyphae of AM fungi. The spores of members of this genus consist of three wall layers. The ontogenetic development of spores of *Pacispora* species is by the formation of a uniform, plate-like germination shield on the surface layer of the inner germination wall. A germ tube grows from this shield and penetrates through the spore wall. The mycorrhizae consist of arbuscules, vesicles, intra- and extra-radical hyphae, as well as of auxiliary cells. The arbuscules, vesicles and hyphae morphologically resembled those of *Glomus* species and stained intensively in trypan blue. The auxiliary cells occur both outside and inside roots and are knobby.


**Etymology:** Resembling "*Glomus*" with identical spore morphotypes.

Spores of species of the genus *Paraglomus* develop blastically at the tip of extraradical hyphae. The spores of the known species of this genus occur singly in the soil. They are globose to irregular and colourless to pale coloured. The sub cellular structure of spores of *Paraglomus* consists of a spore wall comprising two to three layers continuous with those of their subtending hyphae. Spores of *Paraglomus* species germinate by germ tubes emerging from both the lumen of the subtending...
hypha and the spore wall (Morton and Redecker, 2001). Arbuscules of Paraglomus species are cylindrical or slightly flared trunks with branches progressively tapering in width towards the tips (Morton, 2002; Morton and Redecker, 2001). The mycorrhizae of Paraglomus species do not contain vesicles and their intraradical hyphae are frequently coiled within and between cortical cells. The main visible evidence of mycorrhizae of Paraglomus species is their light staining or the lack of any staining reaction in trypan blue or other stains.


Etyomology: Greek, "archaios" = ancient, referring to the ancient position of this genus in Glomales.

Archaeospora is dimorphic, forming both acaulosporioid and glomoid spores (Morton and Redecker, 2001; Sieverding and Oehl, 2006; Spain et al., 2006). Acaulosporioid spores develop laterally, directly on the neck of a sporiferous saccule and are sessile. Two-layered glomoid spores origin blastically at the tip of or intercalary in fertile hyphae, as spores of Glomus species. Germination of Archaeospora spores is by a germ tube emerging from an irregular germination structure (Spain, 2003). Mycorrhizae of Archaeospora (1) do not contain intraradical vesicles or they form rarely, (2) have intraradical hyphae with many coils located within and between cortical cells, (3) stain lightly or not at all in trypan blue and other stains, and (4) are patchily distributed along roots (Morton, 2002).

Spores of *Diversispora* develop blastically at the tip of cylindrical to slightly flared sporogenous hyphae continuous with extraradical hyphae of AM fungi. The mycorrhizae of most *Glomus* species consist of arbuscules, vesicles and hyphae staining intensively in trypan blue whereas those of *D. spurca* lack vesicles and stain variably, from almost no staining to intensive staining (Morton, 2002).


Spores occur singly in the soil or inside roots (Blaszkowski *et al.*, 1998; Sieverding and Oehl, 2006). The spores develop inside the neck of a sporiferous saccule directly at or at a short distance from the saccule originating from the neck. The sporiferous saccule originate terminally or intercalary inside extra- and intra-radical hyphae by their swelling. The spores are globose to subglobose and coloured; their sub cellular structure consists of a multilayered, coloured spore wall and one inner 3-layered, colourless germination wall. In spores lacking the sporiferous saccules, two opposite cicatrices resembling small rings with a slightly raised border are visible. The cicatrices are frequently accompanied by stalks developed from the permanent spore wall layers. The mycorrhizae of *Entrophospora* showed intense staining in trypan blue (Sieverding and Oehl, 2006).
Species of the genus *Ambispora* are dimorphic producing both acaulosporoid and glomoid spores i.e. spores originating similarly to those of *Acaulospora* and *Glomus* species (Morton and Redecker, 2001; Spain et al., 2006). The acaulosporoid spores occur singly in the soil and the glomoid ones are formed singly or in loose clusters in the soil and develop terminally from the thin walled hyphae grown from either the wall of a pedicel or branched germ tubes (Spain et al., 2006). In contrast to the sessile acaulosporoid spores of the genus *Acaulospora* and *Archaeospora*, those of *Ambispora* species develop blastically at the tip of a short branch formed at the distal end of the neck of a sporiferous saccule. This branch is called appendix or pedicel. The sporiferous saccule of *Ambispora* species originate terminally from mycorrhizal extraradical hyphae by their swelling. The spores of the known species of *Ambispora* are globose to subglobose and coloured. The sub cellular structure consists of three layered, coloured spore wall and two inner colourless germination walls. The outer spore wall completes development subsequent to the formation of the outer layer of the first inner germination wall. The spore wall and the outer layer of the first inner germination of the spores of *Ambispora* species are continuous with the pedicel wall layer. The mycorrhiza of the species *Ambispora* consists of arbuscules, vesicles as well as intra- and extra-radical hyphae. All these structures stain faintly in trypan blue (Spain et al., 2006).

Archaeospora trappei the only member of the genus Archaeospora is a dimorphic fungus, producing both acaulosporoid and glomoid spores (Morton and Redecker, 2001; Sieverding and Oehl, 2006; Spain, 2003; Spain et al., 2006). Acaulosporoid spores develop laterally on the neck of a sporiferous saccule, are sessile similarly as most spores of the genus Acaulospora. Two layered glomoid spores originate blastically at the tip of intercalary in fertile hyphae as spores of Glomus species. The sub cellular structure of acaulosporoid spores Archaeospora trappei comprises of a spore wall and one inner germination wall, each consisting of two to three layers. Germination of Archaeospora trappei spores is by germ tube emerging from an irregular germination structure (Spain, 2003).

Mycorrhizae of Archaeospora trappei 1) do not contain intraradical vesicles or they form rarely, 2) have intraradical hyphae with many coils located within and between cortical cells 3) Stain lightly or not at all in trypan blue and other stains and 4) are patchily distributed along roots (Morton, 2002).

SECTION III- LIFE CYCLE OF ARBUSCULAR MYCORRHIZAL FUNGI:

The life cycle of an AM fungus can be divided in three main stages:
A. Establishment of symbiosis: This involves propagule activation, host search, appressorium formation, root penetration and formation of arbuscules.

B. Vegetative growth phase: This involves intra- and extra-radical mycelial growth, increase of fungal biomass, formation of mycelial structures and expansion of mycorrhizal colonization within and between plants.

C. Reproductive phase: This involves the formation of reproductive structures. Resting spores are the major type of propagule.

The AM fungal mycelium is dimorphic and non-septate or coenocytic (Mosse, 1981). The non-septate hyphae allow a fast cytoplasmic flow in a bi-directional way, not only carrying resources from source to sink regions of the fungal colony and/or symbiotic root, but also transporting fungal organelles, such as mitochondria and nuclei (Bago et al., 1998b; Bago et al., 1999).

The major mycelial structures are characterized as follows:

**Intraradical hyphae:** Intraradical hyphae consists of inter- and intra-cellular hyphae in roots that contain storage materials and take part in transportation of substances absorbed by extraradical hyphae from the soil to arbuscules or directly to root cells of the host plant (Bieleski, 1973). Intraradical hyphae may be straight or with H- or Y-shaped branches. They may also form coils, whose frequency of occurrence depends on their location in root and the generic affiliation of the AM
fungal species (Morton, 2000). Generally, coils more abundantly occur at entry points. Intraradical hyphae of *Glomus* species are infrequently coiled in the other regions of a mycorrhizal root. In contrast, coils produced by species of the other genera of AM fungi are abundant and evenly distributed along mycorrhizal roots.

**Arbuscules:** Arbuscule is a specialized morphological structure hypothetically shared by all AM fungal species (Morton and Benny, 1980). Arbuscules are haustoria-like structures that are formed by profuse dichotomous hyphae branching after penetration into inner plant cortical cell walls, forming an interface between fungal tissue and the plant plasma membrane. This interface is thought to be the major site for nutrient and carbon exchanges between both partners, and it is considered the key structure for establishment of a functional symbiosis (Smith and Read 1997; Harrison, 1999). The arbuscules are usually short-lived (1 to 3 weeks), and are preferentially found in young thin roots during early stages of root colonization (Mosse, 1981; Smith and Read 1997; Harrison, 1999). However, long-lived arbuscules have also been reported in woodland plants (Brundrett and Kendrick, 1990). The arbuscule formation is genetically controlled by the host plant, and the numbers of arbuscules formed is dependent on plant species, availability of nutrients, as well as on the fungal partner (Harrison, 1999). Arbuscules are the main sites of nutrient exchange between a plant host and a fungus (Gianinazzi *et al*., 1979). They are formed within the cells of the inner root cortex (Mosse, 1973) and are indicators of active mycorrhizae. Arbuscules differ in morphology, depending on the generic
affiliation of the AM fungal species (Morton, 2000). Fungi of the genera *Acaulospora, Ambispora, Appendicispora, Diversispora, Entrophospora, Glomus, Intraspora, Kuklospora* and *Paraglomus* produce arbuscules with cylindrical or slightly flared, narrow trunks, whose branches progressively taper in width towards tips. Arbuscules of members of the genera *Gigaspora* and *Scutellospora* generally have swollen trunks with branches tapering abruptly at tips (Morton, 2000).

**Types of arbuscules:**

Gallaud (1904, 1905) surveyed microscopically endomycorrhizas of many plant species and divided them into the following four classes based on types of internal fungal structures and named after plant species or plant taxa in which the type structures were found.

1. *Arum maculatum series* (*Arum type*) in which initial fungal penetration into epidermis and hypodermis is followed by development of hyphae along the cortical intercellular airspaces and then penetration of cortical cells to form simple and terminal intracellular arbuscules, the walls of which are modified by becoming very thin and which have an amorphous chitin deposition (Bonfante- Fasolo *et al*., 1990).

2. *Paris quadrifolia* (*Paris type*) in which the fungus is entirely intracellular with irregular coiled hyphae on some of which are formed ‘composite’ (compound arbuscules) that are not terminal and are localized in definite layers. In paris type, both hyphal coils and arbusculate coils are involved in
nutrient exchange, and the surface area of hyphal coils is equal to that of arbuscules in arum type mycorrhizas (Dickson and Kolesik, 1999) as well as by the presence of a membrane and interfacial matrix around hyphal coils and arbusculate coils (Armstrong and Peterson, 2002). Arbuscule and arbusculate coils are separated from the cortical cell cytoplasm by a periarbuscular membrane and interfacial matrix material, both derived from the plant symbiont (Armstrong and Peterson, 2002). The Arum type has been reported to be abundant in agricultural crops where as the Paris type as been found to be more frequent in plants in natural ecosystems (Smith and Smith, 1997; Yamato and Iwasaki, 2002; Ahulu et al., 2005; Tsuyuzaki et al., 2005).

3. **Hepatic (liverwort) series**, resembling Paris type but with arbusculate structures not organized in layers. Gallaud observed this type in gametophytes of *Pellia epiphylla* and *Conocephalum* (Fegalleta) *conicum*.

4. **Orchid series**, in which the fungus is intracellular and tightly coiled, forming ‘pelotons’.

**Vesicles**: Globose or ovoid, thin-walled vesicles are storage organs filled with lipids and glycolipids (Mosse, 1981). They are formed by an intercalary or terminal swelling of hyphae of AM fungi. Vesicles may be inter- or intra-cellular and may be found in both the inner and the outer layers of the cortical parenchyma. In *Glomus* species, vesicles generally are ellipsoid, whereas those of *Acaulospora*, *Entrophospora* and *Kuklospora* highly vary in shape and frequently have knobs and
concavities on their surface (Morton, 2000). Vesicles are never produced by members of the genera *Gigaspora* and *Scutellospora*. No vesicles are found in any species of *Archaeospora*, *Ambispora* and *Intraspora*.

**Auxiliary cells:** Auxiliary cells are formed by short ramifications occurring simultaneously at both sides of the extraradical hyphae. Each ramification generates several branches that swell and form clusters, which are composed of two to more than 20 balloon-like structures, of about 12-39\(\mu\)m in diameter. Auxiliary cells are metabolically active structures, rich in nuclei, organelles and lipids (Jabaji-Hare et al., 1986; Bonfante and Bianciotto, 1995). However, little is known about their biological function. It has been suggested that auxiliary cells are reminiscent of relict reproductive spores (Morton and Benny, 1990) and has been found only in *Gigasporaceae*. They have a spine or smooth surface in *Gigaspora* and *Scutellospora*.

**Extraradical hyphae:** Extraradical hyphae significantly increase the absorptive area of roots (Bieleski, 1973) and form hyphal bridges transferring nutrients between co-occurring plants (Newman, 1988). They are also important fungal propagules colonizing plant roots (Jasper et al., 1989, 1991). The extraradical mycelium is of key importance for the fungus and to the function of the symbiosis. The majority of spores are formed in the external mycelium. The extraradical hyphae are also responsible for spreading the root colonization within and between plants, generating
an underground link between plants in a community (Smith and Read, 1997). They found evidence for distinct intra- and extra-radical mycelium development strategies which could be related to taxonomic differences between the families, and the results were independent of the host plant used.

**Spores:** Spores are multinucleate single cells mainly produced blastically at the tip of extraradical hyphae. Sometimes spores also occur inside roots (Koske, 1985), on the soil surface (Berch and Fortin, 1983), and on plants or their decaying fragments (Blaszkowski *et al.*, 1998). Arbuscular mycorrhizal fungi form spores ranging from 22 to 1050 µm in diameter (Schenck and Perez, 1990). The number of spores produced depends on the fungal species (Blaszkowski, 1993), the plant species and its variety (Blaszkowski, 1993; Hetrick and Bloom, 1986), soil fertility and fertilizer application (Hayman, 1970), host phenology (Giovannetti, 1985), light intensity (Daft and El Giahmi, 1978) and competitive abilities of co-occurring AM fungal species (Gemma *et al.*, 1989). The reproduction of AM fungi is stated to be clonal (Morton, 2000) and the role of spores is to sequester the genetic information of a given fungal species, disperse the information to new habitats, and initiate new individuals spatially separated from the parent organisms (Morton, 1993). Because many components of the subcellular structure of spores are stable in different environmental conditions, they are the most important structures considered in classification of AM fungi.
Spore formation: The genera of AM fungi are separated on the basis of asexual (anamorphic) spore formation and spore characteristics. The term azygospore or chlamydospore describe the anamorphic stage of AM fungi. Spores of AM fungi are multinucleated and are heterokaryotic. The spores can germinate at different times and some taxa reproduce sexually by forming zygospores if proper mating types are present (Tommerup, 1987).

Arbuscular Mycorrhizal fungi can produce ectocarpic spores free in the soil or in sporocarps, also in dead animals (Rothwell and Victor, 1984), in dead seeds (Taber, 1982), in plant roots (Morton and Walker, 1984; Koske, 1985), in dead spores of other AM fungi (Koske, 1984) or in soil surface (Berch and Fortin, 1983a; McGee, 1986). Spores in the soil may be produced terminally or laterally on subtending hyphae or on a single suspensor cell. The sporocarpic species produce spores in loose arrangement or in a highly ordered arrangement around a hyphal plexus (Gerdemann and Trappe, 1974; Berch and Fortin, 1983a; McGee, 1986). Spores in *Glomus* are formed terminally on one or more hyphae. *Glomus* species form single spores or spores in sporocarps where the spores are arranged randomly in the matrix hyphae around the central plexus of sterile hyphae (Gerdemann and Trappe, 1974).

*Acaulospora* and *Entrophospora* tend to form spores associated with a small hyphal chamber. Spores in *Acaulospora* are formed laterally on the stalk of a large terminal and thin walled hyphal chamber (Berch, 1985). However in *Entrophospora,*
spores are produced completely within the neck of the hyphal chamber (Ames and Schneider, 1979).

*Gigaspora* and *Scutellospora* are separated from other AM fungi by mode of spore germination. In *Scutellospora* germination is via the germination shield found within the spore. In *Gigaspora*, no germination shield is formed, and germination is by the direct growth of one or more germ tubes through the spore wall (Walker and Sanders, 1986; Walker, 1987).

**Spore germination:** Spores of AM fungi are able to germinate and grow from a quiescent state in response to different edaphic and environmental conditions, irrespective of the presence of host plants. Germ tubes are not capable of extensive hyphal development, and in the absence of the host, cease growth within 15-20 days of germination (Becard and Piche, 1989; Logi et al., 1998). The elongating germ tubes give rise to a coenocytic mycelial network, containing many nuclei and total mycelial lengths range from 30-50mm in *Glomus caledonium* to 8mm in *Glomus clarum* and to 18-25mm in *Gigaspora margarita* (Louis and Lim, 1988; Gianinazzi-Pearson et al., 1989).

**Spore morphology:** Dissecting, light and electron microscopes are used to determine spore size, shape, colour and wall structure of all known species of AM fungi. *Glomus tenue* is the smallest spores with an average 10-12μm in diameter. In
contrast, *Gigaspora gigantea* is the largest spores, the spore diameter ranged from 183-500 x 291-812μm. Morton (1986) suggested that the variation in spore shape may be the result of environmental stress. Thin walled spores produced in the root cortex are often ellipsoid as in *Glomus intraradices*, *Glomus diaphanum* and *Scutellospora pellucida*. Spores that are produced with a large thick wall are mostly globose or subglobose as in *Glomus clarum* and *Glomus manniihot*. Ovoid, obovoid, pyriform, irregular, reniform, pyriform and clavate spores occur in AM fungi. Spore colour ranged from hyaline to white to pale yellow, orange, red, brown, dark brown or black. The difference in colour could be due to pigmentation in the spore wall or in the spore content (Morton, 1988).

Traditionally, morphological characteristics of the spore walls of AM fungi are used in species identification (Mosse and Bowen, 1968; Gerdemann and Trappe, 1974; Trappe, 1982). Spore wall characteristics became the most important morphological characteristics after Walker (1983) suggested use of standardized terminology for wall murographs. He originally proposed four wall types, viz., the unit, laminated, evanescent and membraneous wall and also introduced the concept of hyphal peridial wall. As new species were described, new categories of spore walls were added, i.e coriaceous wall (Walker, 1986), amorphous wall (Morton, 1986) expanding wall (Berch and Koske, 1986) and germinal wall (Spain et al., 1989).
**Subtending hyphal morphology:** The spores of AM fungi are produced on one or more subtending hyphae. Most *Glomus* species have single subtending hyphae on their spore (Morton, 1988). Some species such as *G. glomerulatum*, *G. heterosporum* and *G. lacteum* produce spores on 1-3 subtending hyphae. *Glomus formosanum*, *G. multisubtextsum* and *G. multicaule* form spores on 1-4 subtending hyphae.

The colour of the subtending hyphae in most species of *Glomus*, *Gigaspora* and *Scutellospora* is similar or lighter than spore wall colour. Subtending hyphal shape ranged from straight, recurved, cylindrical, flared, funnel shaped, constricted to irregular. The opening between the subtending hyphae and the spore content may remain open or may be closed by a plug or septum by spore wall thickening or by spore inner wall. Width and length measurement of the subtending hyphae are used in species identification.

**Sporocarp morphology:** Sporocarps are formed in peridial and possibly in glebal hyphae in some species of *Glomus*. Sporocarps are not known in *Entrophospora*, *Gigaspora* and *Scutellospora* (Berch, 1985; Walkers and Sanders, 1986). Sporocarps are typically absent in *Acaulospora*, the exceptions being *A. myriocarpa* in which spores are in cluster (Schenck et al., 1986) and *A. sporocarpa* which has an aggregation of spores in a network of hyphae (Berch, 1985). External sporocarp colour ranges from white to brown. However, internal sporocarp colour ranges from white to black to brown in *Glomus* species. Sporocarp is irregular but is globose,
subglobose or ellipsoid in *Glomus* species (Morton, 1988). Two terms are used to describe hyphal arrangement in sporocarp: the term peridium is used when the hyphae form a loosely or tightly interwoven network on the surface of the sporocarp, and gleba is used when the hyphae form a matrix in the sporocarp.

**Spore dispersal:** Arbuscular mycorrhizal fungi depend on passive means of spore dispersal. Wind and animals are good vectors for spore dispersal (Friese, 1984; MacMohan and Warner, 1984). In arid ecosystems, wind might be the most important dispersal agent for AM inoculum (Warner et al., 1987). However, animals are the major vectors for AM inoculum in mesic habitats (Maser et al., 1978; Allen, 1987). In soil, spores of AM fungi are protected by tiller or rhizome leaves and scales, which carry spores with them to new sites. When roots arise at the node of the tillers or the rhizomes, hyphae of germinated spore penetrate the young roots and establish mycorrhizal association (Gemma, 1987).

The number of AM fungal spores in the soil is a good indicator of species abundance. Arbuscular mycorrhizal fungal spores are usually more numerous and diverse in cultivated soils than under natural vegetation (Hayman, 1978; Hayman and Stovold, 1979). Since AM fungal spores are not easily dispersed from the point of sporulation, the upper ten centimeters of soil is the best indicator of AM fungal populations (Friese and Koske, 1991).
**Spore germination:** Spore germination in AM fungi has been studied in only a few species. Mosse (1970) studied spore germination in *Acaulospora laevis* where spore germination occurred by the growth of a germ tube from a peripheral chamber that has formed between the walls. Further studies showed that chamber was not formed before germination (Walker, 1987). Berch and Fortin (1983b) studied spore germination in three genera (*Endogone, Gigaspora* and *Acaulospora*) and suggested that there is a phylogenetic relationship between these genera based on the formation of an inner wall germ tube.

There is no published information on spore germination for *Entrophospora* (Walker, 1987). In *Glomus* species, spores typically germinate either by a germ tube that penetrates through the subtending hyphae or by germ tubes that emerge directly through spore wall (Walker and Rhodes, 1981; Miller and Walker, 1986).

**Morphological Characteristics:** Spores, subtending hyphae and sporocarp morphology are used as taxonomical features in AM fungal identification. However, Hall (1977) and Morton (1985) concluded that the characteristics (spore size, spore colour and spore structure) and ontogenetic characteristics (production of arbuscules, vesicles and number of spores) were not influenced significantly by the host. Later, Morton (1988) apparently contradicted himself and hypothesized that either the host or environmental factors may cause variation in morphological structures. Abbott (1982) developed a key for 10 species of AM fungi that was based entirely on the
morphological anatomy of hyphal development in AM fungi and used twenty characteristics including hyphal diameter, mode of branching vesicles, arbuscules and staining reaction in the key and these characteristics are stable in different host and soil environment.

**Molecular Biology:** The biochemical and genetic characterization of AM fungi has been hindered by their biotropic nature, which impedes laboratory culturing. The obstacle had recently been surpassed with the use of hairy root cultures. The first mycorrhizal gene to be sequenced was the small subunit SSU rRNA (ribosomal RNA) (Simon et al., 1992). This gene is highly conserved and commonly used in phylogenetic studies. The SSU rRNA was isolated from spores of each taxonomic group and amplified using PCR techniques (Simon et al., 1993). A molecular clock approach based on the substitution rates of SSU sequences was used to estimate the time of divergence of the AM fungi. The molecular analysis found that the AM fungi are between 353 and 462 million old (Simon et al., 1993). More recent molecular clock analyses date back the origin of AM fungi and first land plants but all data known suggests that AM fungal symbiosis may have been instrumental in the colonization of land by plants.
SECTION IV- ROLE AND BENEFITS OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI:

In terms of ubiquity and partnerships throughout the plant kingdom, mycorrhizal relationships are the most significant plant-microbe symbiosis. It is well documented that elevation of soil P concentrations as a result of intensive agriculture can decrease the soil populations of AM fungi (Smith and Read, 1997). Thus, mycorrhizal technology becomes an important consideration in low-input, organic or soil less agriculture. Plant growth can also be inhibited as a result of the accumulation of phytotoxic levels of heavy metals and organic xenobiotics. Application of mycorrhizal technologies requires knowledge of biodiversity across and within the species involved. This may be achieved through bioaugmentation by inoculating soils with AM fungi or by using transplanted seedlings that already have the appropriate AM fungi in their roots.

Contribution of AM fungi to soil health: Arbuscular mycorrhizal fungi are essential components of soil biota, as they can be found in nearly all ecological situations, mainly in natural ecosystems, particularly those supporting plant communities with high species diversity and in normal cropping systems, especially with sustainable practices (Gianinazzi and Schuepp, 1994). Arbuscular mycorrhizal fungi are obligate symbionts and their life cycle depends on plant roots and in return they decrease disease in the host and reduce population levels of pathogenic
microorganisms in the soil, especially when the supply of P is limiting (Linderman, 1994).

Arbuscular mycorrhizal fungi develop intensively inside roots and within the soil by forming an extensive extraradical network and this help plants considerably in exploiting mineral nutrients and water from the soil. The important role of soil mycelium in the formation of water stable soil aggregates is well documented (Miller and Jastrow, 2000). Arbuscular Mycorrhizal fungi produce a very stable hydrophobic glycoprotein, glomalin, which is deposited on the outer hyphal walls of the extraradical mycelium and on adjacent soil particles, which appear to act as a long-term soil-binding agent (Wright and Upadhyaya, 1999).

**Interaction of AM fungi and abiotic factors:** A number of abiotic factors such as climate change, drought stress, pollution and heavy metal contamination can influence the development of mycorrhizal relationships. Inefficient mining processes, treatment of soil with sewage sludge or industrial effluents, overuse of heavy metal containing fertilizers or gas exhaust are other factors that contribute to the creation of large areas contaminated by heavy metals radionuclides and persistent organic pollutant (Jeffries *et al.*, 2003). Arbuscular mycorrhizal fungi also have a potential role in monitoring the site toxicity (Gucwa Przepiora and Turnau, 2001) and in restoration techniques (Orlowska *et al.*, 2002). A well developed mycorrhizal symbiosis enhance the survival of plants in polluted areas by better nutrient
acquisition, water relations, pathogenic resistance, phytohormone production, contribution to soil aggregation, amelioration of soil structure and also in bioremediation (Jeffries et al., 2003).

Arbuscular mycorrhizal fungi have been found to decrease cesium uptake by plants (Berreck and Haselwandter, 2001) and this could be used in the establishment of plant vegetation on soil contaminated with radionuclides, and therefore have the potential to reduce environmental risks (Jeffries et al., 2003).

**Interactions of Arbuscular Mycorrhizal (AM) fungi and biotic factors:**

Arbuscular mycorrhizal fungi are the key components of soil microbiota and interact with other microorganisms in the rhizosphere (Bowen and Rovira, 1999). Mycorrhizal colonization changes plant physiology and certain nutritional and physical properties of the rhizosphere soil. This in turn, affects colonization patterns by soil microorganisms by the so-called mycorrhizosphere effect (Gryndler, 2000). Arbuscular mycorrhizal fungi thus interact with natural and introduced microorganisms in the mycorrhizosphere hence affecting the soil properties and quality. Soil microorganisms can produce compounds that increase root cell permeability, thereby increasing the rates of root exudation. This in turn stimulates the growth of hyphae of AM fungi in the rhizosphere and facilitates root penetration by the fungus. Rhizosphere microorganisms are also known to affect the presymbiotic stages of AM fungal development (Giovannetti, 2000) such as spore germination and
germ tube growth (Azcon-Aguilar and Barea, 1995). Biologically active substances such as amino acids, plant hormones, vitamins, other organic compounds and volatile substances (CO₂) produced by soil microorganisms can stimulate the growth rates of AM fungi (Azcon-Aguilar and Barea, 1995).

In Horticulture and Agriculture: The use of AM fungi in agriculture could lead to a considerable decrease in the amount of chemical pollution in soil water, as recently demonstrated for maize (Giovannetti, 2001). This clearly indicates the potential of AM fungi for promoting a low chemical input agriculture. The recent development in molecular probes could differentiate AM fungi within roots and soils (Jacquot-Plumey et al., 2001) and also opened new biotechnological perspectives for defining their population biology and management strategies in the use of these symbiotic microbes in agriculture. Successful inoculation is usually achieved when AM fungi are introduced very early in the plant developmental process followed by the use of low amount of phosphate fertilizers and selective use of pesticides (Guillemin et al., 1993). By doing so, colonization by AM fungi will follow root development of the inoculated seedlings or cuttings with the consequence that plants will already be extensively mycorrhizal when transplanted into the field. Micropropagated plants inoculated with AM fungi can 1) reduce plant losses during the acclimatization phase, 2) subsequently stimulate plant development (induce flowering) and 3) increase productivity after transplantation to the field (Estaun et al., 1999).
**In alleviating desertification:** As a result of the ecosystem degradation processes in desertification of threatened areas, disturbance of natural plant communities is often accompanied by loss of physico-chemical and biological properties, such as soil structure, plant nutrient availability, organic matter content and microbial activity. There is an increasing interest in using AM fungi to improve revegetation processes for desertified ecosystems as inoculants. Experiments carried out for assessing the long-term benefits of inoculation of shrub legumes with rhizobia and AM fungi include improving the establishment of target legume species as well as the benefits induced by symbiotically tailored seedlings in physico-chemical properties of soil (Requena *et al.*, 2001).

**In bioremediation of soils containing pollutants:** Phytostabilisation is a process in which pollutants are immobilized by plant activity, resulting in attenuation of wind and soil erosion and run off processes into ground water or air (Losi *et al.*, 1994). Phytodegradation covers the whole range of metabolic processes in which plants usually assisted by microorganisms, degrade organic compounds such as hydrocarbons, pesticides and explosives. Phytoextraction involves hyper metal accumulating plants, which contain more than 1\% of metals in harvestable tissues. Mycorrhizal fungi were shown to be involved in the degradation of organic pollutants and thus may be potentially useful in phytodegradation. Although colonization by AM fungi was negatively affected by increasing polycyclic aromatic hydrocarbons
(PAHs) level in soil (Levyal and Binet, 1998) enhanced plant survival and growth by decreasing P deficiency (Joner and Levyal, 2001), water stress (Sanchez-Diaz and Honrubia, 1994), improving membrane integrity (Graham et al., 1981) and stimulation of oxidative enzyme production (Salzer et al., 1999).

Arbuscular mycorrhizal fungi can also be helpful in the management of constructed wetlands used for detoxification of a broad range of toxic substances. This plays an important role in the initial steps of the establishment of wetland places and subsequently influences plant biodiversity in later stages, encouraging the re-appearance of mycorrhizal species (Vangronsveld et al., 1996). Recently, the presence of AM fungal symbiosis was also demonstrated in hyper accumulating plants, which is being used in phytoextraction. For successful bioremediation, symbionts must be selected that could withstand the hostile environment of polluted sites (Jeffries et al., 2002).

**Phytoremediation:** The use of AM fungi in ecological restoration projects have enabled their host plant establishment on degraded soil and improved soil quality and health (Jeffries et al., 2002). A relatively new approach to restore land and protection against desertification is to inoculate the soil with AM fungi with the reintroduction of vegetation. Jeffries et al. (2002) demonstrated that significantly long term improvement in soil quality parameters was attained when the soil was inoculated with a mixture of indigenous AM fungal species compared to uninoculated soil and
soil inoculated with a single exotic species of AM fungi. The benefits observed were an increased plant growth and soil nitrogen content, higher soil organic matter content and soil aggregation. The improvements were attributed to the higher legume nodulation in the presence of AM fungi, better water infiltration and soil aeration due to soil aggregation. Inoculation with native AM fungi increased plant uptake of P thereby improving plant growth and health, and also supported AM fungi as a biological tool in the restoration of self-sustaining ecosystems (Jeffries et al., 2002).

**Soil quality**: Arbuscular mycorrhizal fungi enhance soil aggregate stability through the production of a soil protein known as glomalin. Glomalin related soil proteins (GRSP) have been identified using a monoclonal antibody (Mab32B11) raised against crushed mycorrhizal spores and is defined by its extraction conditions and reaction with the antibody Mab32B11. Glomalin is hypothesized to improve soil aggregate water stability and decrease soil erosion (Rillig, 2004).

The AM fungal diversity regulates patterns of plant diversity, if one AM fungal species or indigenous species becomes extinct in a habitat (Allen et al., 1995). As AM fungal species are below ground organisms, they spread slowly over short distances, using plants as stepping-stones. When a plant establishes itself, the plant and soil dwelling mycorrhizal community coadapt to develop a symbiosis (Allen et al., 1995). Moreover, plants in distributed areas of low fertility would benefit from a diverse mycorrhizal community, as there is greater chance of adaptation to
environmental changes (Abbott and Gazey, 1994). Additionally soil surface temperature above 60°C kill mycorrhizal spores which decreases the infectivity potential in the surface layer (Thompson, 1989).

**Crop nutrition**: Arbuscular Mycorrhizal (AM) fungi play a significant role in crop nutrition, by increasing total P uptake (Koide et al., 2000), growth and yield (Ibibijen et al., 1996; Koide et al., 2000) but under conditions of high soil P concentrations, it may reduce crop growth (Kahiluoto et al., 2001). Though P uptake usually dominates consideration of the AM fungal association, it has become increasingly apparent that mycorrhizae can be important in the uptake of other nutrients. Zinc is most commonly reported being influenced by the AM association, though uptake of Cu, Fe, N, K, Ca and Mg also being enhanced by AM fungal association (Smith and Read, 1997; Clark and Zeto, 2000).

**Crop pests and diseases**: Arbuscular mycorrhizal fungi play a major role in the suppression of crop pests and diseases, particularly soil-borne fungal diseases (Linderman, 1994; Borowicz, 2001). Other types of pest and disease causing organisms which may be suppressed by AM fungi include pathogenic nematodes (Talavera et al., 2001) above ground fungal diseases (Feldmann and Boyle, 1998) and herbivores (Gange et al., 2002). Though the mechanisms involved are complex,
change in nutritional status resulting in changes to leaf defensive chemicals, are likely to be involved in above ground interactions with herbivores (West, 1995).

**Interaction with other soil microorganisms:** Bacterial communities and specific bacterial strains promote germination of AM fungal spores and can increase the rate and extent of root colonization by AM fungi (Johansson et al., 2004). The legume—Rhizobium symbiosis is strongly influenced by AM fungi and enhanced P nutrition arising from the AM fungal colonization resulted in an increase in nodulation and N₂ fixation (Vazquez et al., 2002).

**Crop water relations:** Arbuscular mycorrhizal fungi increase the host plant's tolerance to water stress (Auge, 2004) and several mechanisms have been proposed to explain the effect including increased root hydraulic conductivity, improved stomatal regulation, osmotic adjustment of the host and improved contact with soil particles through the binding effect of hyphae, enabling water to be extracted from smaller pores (Auge, 2004).

**Benefits of AM fungi:** Arbuscular Mycorrhizal colonized plants exhibit an increased rate of photosynthesis (Dixon et al., 1994) and tolerance to drought (Osnubi et al., 1992) and salinity (Rosendahl and Rosendahl, 1991). Resistance to root pathogens also increases primarily due to AM fungi occupying the root niche and
increased plant vigour (Smith and Read, 1997). Another feature important in the establishment of seedlings is the transport of photo derivatives from an unshaded to a shaded plant via the AM fungal mycelium (Eissenstat and Newman, 1990).

As AM fungi is a mutualistic symbiont, it drains upto 20% photosynthetic carbon (Jakobsen and Rosendahl, 1990) and in return, provide plants with large amounts of nutrients (P, N, K, Zn) and water from the soil. Arbuscular Mycorrhizal fungi also produce glycoprotein extracellularly on the mycelia in the bulk soil, which together with the physical network of hyphae helps to aggregate soil thus improving aeration and water percolation (Wright and Upadhyaya, 1998). The carbon inflow to soil attracts soil microbes; altogether producing a functionally diverse and dynamic soil biota which are fundamental for plant nutrition in natural systems and sustainable agriculture (Schreiner et al., 1997).

The benefit of AM fungal symbiosis depends on when in a plant life stage its roots are colonized by AM fungi (Solaiman and Hirta, 1996). The outcome is also affected by the growth rates of both fungi and plant. Moreover, the AM symbiosis is also influenced by the composition of plant and fungal species (Schreiner et al., 1997) by the hierarchical structure of AM fungal species in the root niche, and by their inherited genetic and functional diversity (Smith and Gianinazzi Pearson, 1988). Additionally the outcome of an AM fungal symbiosis is affected by soil properties,
soil and plant treatments and the presence and amount of soil microbes being mutualists, commensalists and inhibitors or parasites.

Mycorrhizae also enhances plant growth and improves crop yield by essential nutrient absorption, improvement in plant photosynthesis, nutrients translocation and plant metabolism processes which reduces the use of chemical fertilizers and in turn increases income for the farmers. Mycorrhizae are endurable to several chemical substances; e.g. pesticide such as endrin, chlordane, methomyl carbofuran, herbicide such as glyphosate, fuazifopbutyl, chemical agents for plant disease elimination such as captan, benomyi, mane6 triforine, mancozed and zineb.

**Evaluation of biodiversity and conservation of AM Fungi:** The diversity of AM fungi has significant ecological consequences as individual species or isolates vary in their potential to promote plant growth and adaptation to biotic and abiotic factors. Thus, the composition and dynamics of populations of AM fungi have marked impact on the structure and diversity of the associated plant communities, both in natural and agricultural ecosystems (van der Heijden *et al.*, 1998). An important pre requisite to the analysis of populations of AM fungi in ecological studies is the correct identification of individual isolates. Allozymes have been helpful in providing diagnostic biochemical markers to identify species of AM fungi, even in colonized roots. However, the most powerful tools to study the evolution and
population genetics of AM fungi are molecular techniques that analyze DNA sequences (Sen and Hepper, 1996).

A wide variety of techniques could be employed to detect DNA sequence variation in populations of AM fungi (Lanfranco et al., 1998). PCR amplification of targeted genomic sequences followed by RFLP, allele-specific hybridization, direct sequencing or single strand conformation polymorphisms are increasingly used to detect AM fungi in natural ecosystems (Redecker et al., 1997; Helgason et al., 1999). DNA markers have been successfully employed to track specific AM fungi from agricultural and natural ecosystems (Jacquot-Plumey et al., 2001).

SECTION V- INFLUENCE OF CLIMATIC FACTORS ON AM FUNGI:

Soil environmental conditions as well as plant nutrient level, light intensity and cropping systems affect the development of AM fungi and the formation of mycorrhizae (Evans and Miller, 1990; Furlan and Fortin, 1977; Jasper et al., 1989, 1991; Reinharts et al., 1994).

**Temperature:** Of the factors influencing mycorrhizal development and function, temperature is important (Fabig et al., 1989). Temperature strongly influences the physiology of living organisms and low temperature influence plant roots and mycorrhizal fungal development. Mycorrhizal development is usually optimal; at least in plants of cool temperate climates at 20–25°C (Matsubara et al., 2000; Zhang
et al., 1995) and maximal spore germination occurs between 20°C and 28°C depending on the species (Wang et al., 1997). Charest et al. (1993) reported that mycorrhizae counteract chilling injury in maize (Zea mays L.). AM colonization increased nodule size and leaf N concentration in soybean grown at a root zone temperature of 15°C (Zhang et al., 1995). Mycorrhizal leek plants exposed to the same root zone temperature were better able to absorb P32 from soil than non-mycorrhizal plants (Wang et al., 2002).

There is data on the effect of temperature on spore germination and hyphal elongation under axenic conditions. Whereas Glomus mosseae and Acaulospora laevis can germinate between 10-18°C and 30°C with an optimum between 20°C and 30°C (Safir, 1986), and germination was best at 10-25°C for G. caledonium (Tommerup, 1983) and at 25°C for G. epigaeum (Graham, 1982). The optimal germination temperature seems to depend on the environment where the fungus has been isolated. Scutellospora coralloidea and S. heterogama both isolated in Florida germinated at 34°C whereas G. mosseae isolated from cooler regions showed maximal germination at 20°C and failed to germinate at 34°C (Schenck et al., 1975).

Apart from the direct effect of temperature on AM fungal spore germination and hyphal growth, the temperature to which dormant spores are exposed can affect spore germination (Hepper and Smith, 1976; Gemma and Koske, 1988), spore mortality and hyphal growth pattern (Juge et al., 2002). Cold storage (4°C) of spores
of *Glomus intraradices* for more than 14 days increased spore germination, reduced spore mortality and resulted in the growth of a clearly distinguishable, several centimeter long hypha with few branches, whereas hyphal growth of spores stored at a higher temperature (25°C) was without a viable dominant hyphae, and the hyphae emerging from the spores continuously curled and branched heavily (Juge *et al.*, 2002).

A number of studies reported inhibition of mycorrhizal development at temperatures allowing plant growth. Arbuscular mycorrhizal colonization of soybean was strongly repressed at a root zone temperature of 15°C (Zhang *et al.*, 1995). Colonization of barley was similarly repressed at 15°C and inhibited at 10°C (Baon *et al.*, 1994). Arbuscular mycorrhizal spores and mycelium from 20 glomalean isolates from soil and root debris survived in storage at 80°C (Kuszala *et al.*, 2001). The stimulation of AM spore germination by a period of exposure to low temperature suggests that some AM fungi possess mechanisms of dormancy that synchronize their activity to the seasonal cycle of cool climate ecosystems (Juge *et al.*, 2002). Sporulation by an isolate of *Glomus fasciculatum* was greatest at 30°C, which coincided with the optimum temperature for the host plant (Ferguson and Menge, 1982).

Root colonization by AM fungi often decreases when the temperature exceeds 30°C (Bowen 1987), and soil temperatures above 40°C which are generally lethal to
AM fungi (Bendavid-Val et al., 1997). The germination of spores of *Scutellospora coralloidea* and *S. heterogama* was found to decrease above 34°C (Schenck et al., 1975). The presence of AM fungal arbuscules in soybean roots were found to decrease above 30°C, while production of external hyphae outside soybean roots was found to decrease above 34°C (Schenck and Schroder, 1974). Haugen and Smith (1992) reported that colonization of cashew (*Anacardium occidentale*) roots by *Glomus intraradices* declined above 30°C and was severely reduced at 38°C.

Temperature also can influence secondary mycorrhizal colonization so that certain species might not achieve that minimum level of colonization necessary to trigger sporulation (Frank and Morton, 1994). Temperature is certainly one of the major environmental factors influencing AM fungal species distribution in the field. Schenck and Schroder (1974) observed that sporulation and mycelial growth of *Gigaspora* species was optimized at higher temperatures within the range of temperature tested and correlated the affinity of the fungus for high temperature with its presence and abundance in summer rather than spring or winter crops. Koske (1987) observed that some AM fungal species were more abundant in northern cooler regions than in southern warmer regions within a latitudinal temperature gradient and also found that frequency of some species such as *Scutellospora weresubiae* and *Glomus tortuosum* was correlated with higher temperatures.
Negative effects of mycorrhizae on plant growth at suboptimal temperatures have been attributed to the inability of an established fungus to take up (Bowen et al., 1975) or transport P (Hayman, 1977) while still utilizing host carbon (Hetrick and Bloom, 1986).

**pH**: The efficiency of AM fungi is influenced by properties of the soil (Slankis, 1974). The AM fungi have been found in soils from pH 2.7 to 9.2, but different fungal isolates have varied pH tolerances (Siqueira et al., 1984). Optimal pH conditions for spore germination differ between species and genera. The optimal pH for spore germination seems to be linked to the pH of the soil, where the AM fungi are isolated (Giovannetti, 2000). *Acaulospora* species germinates between pH 4 and 5 (Hepper, 1984), *Gigaspora* species at a pH from 4 to 6 and *Glomus* species between pH 6 and 9 (Green et al., 1976).

**CO₂**: Although released by roots, CO₂ cannot be regarded as a plant specific signal for AM fungi, as CO₂ levels in the soil can also be increased from other sources such as the respiration of soil organisms. When initiating AM fungal monoaxenic cultures, an enriched CO₂ atmosphere developed in the petriplate due to root organ respiration is probably an activator of spore germination and of asymbiotic hyphal growth under these conditions (Vierheilig and Bago, 2005).
The CO₂ effect on AM fungi seems to be concentration dependent. In axenic systems, CO₂ levels ranging from 0.5 to 2.5% stimulate hyphal growth of *Gigaspora margarita* (Becard *et al.*, 1989; Becard *et al.*, 1992) CO₂ level of 0.1% showed no effect on hyphal growth (Poulin *et al.*, 1993) and high CO₂ levels (5%) irreversibly inhibited in vitro growth of *Glomus mosseae* (Le Tacon *et al.*, 1983). Hyphal growth of AM fungi was highest at CO₂ levels around 2% (Becard *et al.*, 1992; Poulin *et al.*, 1993) a concentration that usually is found in most soils. Becard and Piche (1989) suggested that during germination CO₂ may be a net source of carbon for anabolic processes of the spore. Bago *et al.* (1999) have demonstrated recently that a significant rate of trehalose, a short-term fungal storage carbohydrate was $^{13}$C-labelled when $^{13}$CO₂ was supplied to asymbiotic spores.

**Light:** In nature, the exposure of soil borne AM fungi to light is an extremely unlikely event as underground roots are colonized. Light treatments affect the growth pattern of axenically growing hyphae. Light induced hyphal branching was observed in developing germ tubes of *Gigaspora gigantea*, *Gi. rosea* and *Glomus intraradices* (Nagahashi *et al.*, 2000).

Light availability (presumably through its effects on plant carbon fixation) is positively correlated with AM fungal formation (Bethlenfalvay and Pacovsky, 1983; Tester *et al.*, 1986), P uptake (Son and Smith, 1988; Smith and Gianinazzi-Pearson, 1990), root soluble carbohydrate levels (Graham *et al.*, 1982), and plant growth
observed that mycorrhizal colonization in onion roots was reduced and consisted of
fewer arbuscules and external hyphae under lower light intensity.

Earlier studies demonstrated a decrease in growth response on inoculation
with AM fungi as light intensity diminished (or as defoliation increased), but little or
no change in the extent of intraradical AM fungal colonization (Bayne et al., 1984;
Pearson et al., 1991) was observed. Tester et al. (1986) measured fewer AM fungal
entry points under low irradiance, but no change in the rates of either root or AM
hyphal extension. Hayman (1974) noted fewer and smaller arbuscules in AM plants
grown at low light intensity compared to those grown at higher light (Pearson et al.,
1991). Similarly, decreased AM sporulation (Ferguson and Menge, 1982) and fewer
vesicles (Bethlenfalvay and Pacovsky, 1983, Pearson et al., 1991) have been
observed in a variety of herbaceous plants under decreased photon irradiance.

As AM fungus is a significant carbon sink in the mycorrhizal plant system
(Kucey and Paul, 1982; Harris et al., 1985), the influence of light environment on
mycorrhizal colonization is indirectly mediated by the carbon status of the host plant.
Previous studies in AM systems have shown that light intensity decreases root to
shoot ratio (Son and Smith, 1988), root length (Tester et al., 1985) and initiation of
lateral roots (Tester et al., 1986). Several studies have documented lower levels of
soluble carbohydrates in roots (Hayman 1974) and diminished root exudation under low irradiance (Graham et al., 1982).