CHAPTER 2

REVIEW OF LITERATURE
REVIEW OF LITERATURE

Arbuscular mycorrhizae may have been described as early as 1842 (Nageli, 1842). Trappe and Berch (1985) and Rayner (1926 - 1927) cited early observations of the symbiosis during the period 1875–1895. Extensive surveys of host plants and sophisticated anatomical descriptions of what are most certainly arbuscular mycorrhizas are given by Schlicht (1889), Dangeard (1896), Janse (1897), Petri (1903), Gallaud (1905), Peyronel (1924), Jones (1924) and Lohman (1927).

As early as 1889, Schlicht had observed the basic anatomical relationships between host and fungal tissues. Janse (1897) called the intramatrical spores “vesicules” and determined that other structures, named “arbuscules” by Gallaud (1905), were located in the inner cortex. Gallaud (1905) made very accurate observations of the arbuscule and concluded that it is entirely surrounded by a host membrane, which was later confirmed by Cox and Sanders (1974). 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 year’s later (Cox and Sanders, 1974). Gallaud (1905) further distinguished between Arum and Paris types of arbuscules (Smith and Smith, 1997). Jones (1924) described the appresorium. Scannerini and Bellando (1968) first noted that a space between the host membrane and the fungal wall contained materials of host origin, probably unconsolidated components of host cell wall. The recognition that all fungi did not form vesicles led to the proposal that this symbiosis should be renamed as ‘arbuscular mycorrhiza’. In some such symbiotic association, fungi may not produce proper arbuscules (Smith and Smith, 1997).

Early researchers used classical methods of cutting and staining sections of paraffin-embedded roots to produce excellent drawings and photographs of the arbuscular mycorrhiza. Alternatively, very good photographs have been obtained by cutting sections of fresh roots on a freezing microtome. However, both methods are laborious if mycorrhization is to be quantified. The problem was largely solved by clearing the roots of cytoplasm by heating in KOH and staining fungal cell walls with trypan blue in lactophenol (Phillips and Hayman, 1970). The Phillips and Hayman paper is probably among the most frequently cited of all papers dealing with arbuscular mycorrhiza, but the use of hot KOH as a clearing agent was by no means new, having already been used by Janse (1897), Peyronel (1940) and Bevege (1968).
There are now other methods of staining, but the basic procedure of using KOH to remove host cytoplasm is common to nearly all. Although there were some notable reports of the widespread nature of arbuscular mycorrhizas prior to the 1970 publication of Phillips and Hayman (Janse, 1897; Gallaud, 1905; Jones, 1924; Lohman, 1927), the arbuscular mycorrhizal fungi were still considered by most to be rare. With the broad application of clearing and staining, however, arbuscular mycorrhizas were more readily documented in abundance in many habitats (Read et al., 1976). Quantification of mycorrhization has been achieved in various ways. Many early studies simply cut root systems into small pieces and determined the proportion of the pieces that were mycorrhizal.

Probably the most popular method today is based on the line intersect technique devised by Newman (1966), which was possibly first applied to mycorrhizas in 1975 (Sparling and Tinker, 1975). Giovannetti and Mosse (1980) later compared various methods of mycorrhiza quantification, which led to greater acceptance of the line intersect method. Mycorrhizas are complex symbioses and the fungi involved produce a variety of structures within the root. Quantification of these structures (hyphae, arbuscules and vesicles) was standardized by the method proposed by McGonigle et al., (1990).

Although there were already many independent descriptions of the arbuscular mycorrhiza in the late 1800s and early 1900s, the true identity of the fungi involved remained unknown for many decades. So unclear was their identity that at one point the possibility was circulated that a single fungus could form both ectomycorrhizas and arbuscular mycorrhizas (Lohman, 1927). The inability to properly identify a fungus as being arbuscular mycorrhizal was caused in large measure by the inability to independently culture any of them. The classical way to identify an agent of disease (and, by extension, the fungi responsible for the mycorrhizal symbiosis) is to apply Koch’s postulate, and one of the necessary steps is the isolation and culture of the organism involved. From the earliest days there appear to have been attempts to independently culture arbuscular mycorrhizal fungi. Often, researchers attempted to use standard nutrient media, or standard media amended with some “vital component”. Magrou (1946), working in France, observed fungal growth from cut ends of intramatrical hyphae in pieces of surface-sterilized potato roots in hanging drop cultures. The emerging hyphae grew quite vigorously but they could not be subcultured. Their growth stopped when the supporting root piece became moribund.
Stahl (1949), in Germany, found that the arbuscular mycorrhizal fungus could grow 10 cm across sterile sand if it remained attached to a living host, but she too failed at its independent culture. Between 1952 and 1957 a fungus first isolated by Nicholls (1952) from surface-sterilized mycorrhizal onion roots was identified as a strain of *Pythium ultimum*. In 1955 Harrison, also from the Bristol group, isolated this organism again using the hanging drop technique of Magrou. Experiments to test whether inoculation with such isolates could produce typical arbuscular mycorrhizas were summarized by Hawker *et al.*, (1957). The abstract of that paper reads “Inoculation with the isolates of *Pythium ultimum*, under certain conditions led to development of typical hyphae and vesicles within the root and, in older seedlings, to formation of the characteristic arbuscules.” However, this observation was made on roots from open pot cultures, which are subject to soil contamination from adjacent pots.

In 1961, Barrett reported the isolation and culture of fungi from arbuscular mycorrhizal roots via a transitional 146 stage of growth on pieces of hemp seed. He called the fungus *Rhizophagus* and claimed that it produced arbuscular mycorrhizas in other test plants. Re-isolation of the fungus from such plants again required the transitional hemp seed phase. Mosse (1961) once obtained arbuscular mycorrhizas by inoculating a few plants with this fungus in an open pot experiment maintained for a long time, but subsequent tests with better-protected plants failed (Mosse, 1963). Gerdemann (1971) was unable to culture the fungus using the hemp seed technique. Because the arbuscular mycorrhizal fungi could not be cultured, their identities as the fungi responsible for the arbuscular mycorrhizal symbiosis had to be established in other ways. In the 1920s and 1930s Peyronel (1923, 1924, and 1937) traced the hyphae from mycorrhizas to spores of *Endogone fuegiana*, *Endogone vesiculifera* and another *Endogone* species. He also advanced the notion that the typical syndrome of arbuscular mycorrhiza was due to a dual infection by a *Rhizoctonia* and *Endogone*, and this was widely believed at the time. However, he did not test to see if inoculation with any particular fungus resulted in a typical arbuscular mycorrhiza. This was not to happen until Mosse’s first successful “vesicular-arbuscular mycorrhizal infection” of strawberry (Mosse, 1953) using non-sterile sporocarps of a fungus initially named *Endogone mosseae* in her honour (Nicolson and Gerdemann, 1968), which later became *Glomus mosseae*. Incidentally, Mosse’s 1953 publication describing her landmark research consisted of three very brief paragraphs. Inoculation with surface
sterilized sporocarps associated with mycorrhizal strawberry roots also produced mycorrhiza in apple, wheat, various grasses, tomato and lettuce in open pot experiments, demonstrating its wide host range (Mosse, 1956).

Gerdemann (1955) showed that spores from his “type B” isolate, later named *Gigaspora gigantea*, had a wide host range and could successfully form arbuscular mycorrhizas with several species of plants including red clover, maize, strawberry and sweet clover. Gerdemann (1955) carefully noted that the mycorrhiza from his “type B” spores was arbuscular and that no vesicles were produced. It thus became clear that there were at least two patterns of symbiotic development by arbuscular mycorrhizal fungi. According to Lohman (1927) and Kelley (1931) the name mycorrhiza is not correctly used because some hosts of arbuscular mycorrhizal fungi do not house the fungi in true roots at all.

The naming of organisms and the establishment of their evolutionary relationships are of great importance in any field of biology. At the 1974 Leeds meeting (Sanders *et al.*, 1975), the name *Endogone* was used by many in attendance to describe the “phycomycetous endomycorrhizal” fungi. Another outdated name for arbuscular mycorrhizal fungi, *Rhizophagus*, was also in use at the time and continued to be used until about 1977. 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 the soil. No connection to the mycorrhizal symbiosis had yet been suggested. Tulasne brothers considered *Glomus* to be closely related to *Endogone*.

German mycologist, Link (1809) placed the AMF in *Endogone*. Tulasne and Tulasne (1844) described genus *Glomus* comprising two species (*Glomus microcarpum* and *Glomus macrocarpum*). The genus *Sclerocystis* was described by Berkeley and Broome (1873). Fries (1849) established the Endogonaceae, placing it in the Tuberales, but the family was transferred to the Mucorales by Bucholtz (1912). Dangeard (1896) was the first to describe an arbuscular mycorrhiza, which happened to have formed from poplar roots. Thaxter (1922) revised the family Endogoneae, placing all members of *Glomus* in the genus *Endogone*, while maintaining the genus *Sclerocystis*. Bucholtz (1912) placed Endogonaceae in the Mucorales, due to the affinities of *Endogone* with the members of the Mortierellaceae. The family Endogonaceae was placed in its own order, Endogonales, by Moreau (1953), which was later validated by Benjamin (1979). Mosse (1953), Gerdemann (1955, 1961,
1965) and Gerdemann and Nicolson (1962, 1963) added more species to Peyronel’s (1924, 1937) existing list of *Endogone*. Gilmore (1968) further added six spore types E2- E7, found in pot cultures. The name ‘*Rhizophagus*’ was also in use until 1977.

The name “mycorhiza” was given to the peculiar association between tree roots and ectomycorrhizal fungi by Frank (1885). Thorough discussion of the derivation of the word “mycorhiza”, including the incorporation of the second r is given by Kelley (1931, 1950). Frank (1887) recognized a difference between ectotrophic and endotrophic mycorrhizas, which included only ericaceous and orchid mycorrhizas at the time. Both the host plant and the fungus potentially benefit from the association (Powell and Bagyaraj, 1984). Fungal hyphae are efficient in absorbing mineral nutrients, especially phosphate ions, some of which are passed to the host plant (Lewis, 1973). VAM fungi produce two types of mycelial systems: an internal mycelium within the epidermis and cortex of the host roots, and an external mycelium in the soil (Harley and Smith, 1983).

Some genera like *Gigaspora* and *Scutellospora* are arbuscular only and do not form vesicles but form auxiliary cells in the soil matrix. Azygospore or chlamydospore describes the asexual stage of mycorrhizal fungi (Benjamin, 1979). Walker and Sanders (1986) did not believe that there was good evidence for a sexual process in the formation of these spores. They suggest the simple term “spore” to avoid the use of azygospore with its implied “sexual” connotation. They agreed with Koske and Tessier (1983) who questioned the use of the term azygospore for the description of *Gigaspora reticulata*, and with Koske and Walker (1984 and 1985) who dropped the term without comment. Spores are multinucleated and may be heterokaryotic. The spores can germinate at different times, and some taxa reproduce sexually by forming zygospores if proper mating types are present e.g. *Gigaspora decipiens* (Tommerup, 1987). Researchers were encouraged to create a suitable alternative approach to the reproductive structures in VAM fungi. Morton (1988) estimates that 70% of VAM fungi produce ectocarpic spores.

Berch (1986) first used the term ‘sporogenous vesicle’ to describe the hyphal swelling associated with spores of *Entrophospora*. Walker (1987) suggested that the so-called azygospores in *Acaulospora* are sporangiospores and that the saccule is a sporangium. Species of *Gigaspora* and *Scutellospora* form large spores in the soil. Spores are produced terminally on a single, bulbous subtending hypha that is called a
bulbous suspensor cell (Morton, 1988). Nicolson and Gerdemann (1968) used the term azygospore to describe spores in *Gigaspora* because of the similarity of the single subtending hypha to a gametangium in *Endogone*. There is no evidence that spore development in *Gigaspora* or *Scutellospora* is evolved from zygospores via the reduction of one of a pair of gametangia (Powell and Bagyaraj, 1984). In *Gigaspora*, no germination shield is formed, and germination is by direct growth of one or more germ tubes through the spore wall (Walker and Sanders, 1986; Walker, 1987). A unispored sporangium usually has a thinner wall than does a chlamydospore (Hammill, 1981).

VAM fungi can produce spores in dead animals (Rothwell and Victor, 1984), in dead seeds (Taber, 1982), in plant roots (Nicolson and Schenck, 1979; Schenck and Smith, 1982; Morton and Walker, 1984 and Koske, 1985), in dead spores of other VAM fungi (Koske, 1984) or on soil surface (Gerdemann and Trappe, 1974; Tandy, 1975; Berch and Fortin, 1983a; McGee, 1986).

In *Entrophospora*, spores are produced completely within the neck of the hyphal chamber (Ames and Schneider, 1979). Spores in *Acaulospora* are formed laterally on the stalk of a large, terminal, and thin-walled hyphal chamber (Berch, 1985). Both *Glomus ambisporum* and *Glomus heterosporum* form spores in a sporocarp and in a manner similar to *Sclerocystis* (Walker, 1987). The small chamber in *Acaulospora* and *Entrophospora* is termed a mother spore (Mosse, 1970) a vesicle (Gerdemann and Trappe, 1974) a hyphal terminus (Schenck et al., 1984) a soporiferous saccule, a sporogenous saccule (Berch, 1985) or a swollen sac (Morton, 1988).

In *Sclerocystis* spores always form in sporocarps and are arranged around the central plexus of sterile hyphae (Gerdemann and Trappe, 1974). Sporocarps are formed in peridial and possibly in glebal hyphae of *Sclerocystis* and in some species of *Glomus* (Gerdemann and Trappe, 1974). Sporocarps are not known in *Entrophospora*, *Gigaspora*, and *Scutellospora* (Ames and Schneider, 1979; Berch, 1985; Walker and Sanders, 1986). Sporocarps are absent in *Acaulospora*. The exceptions are *A. myriocarpa* in which spores are in a cluster (Schenck et al., 1986) and *A. sporocarpa* which has an aggregation of spores in a network of hyphae (Berch, 1985). Shape of sporocarp is irregular in *Glomus*, but it is globose, sub globose or
ellipsoid in *Sclerocystis* species (Morton, 1988). Sporocarps of diameters less than 1 mm can be separated by wet sieving and centrifugation (Powell and Bagyaraj, 1984).

Berch and Fortin (1983b) studied spore germination in three genera (*Endogone, Gigaspora*, and *Acaulospora*) and suggested that there is a phylogenetic relationship between these three genera based on the formation of an inter wall germ tube. There is no published information on spore germination for *Sclerocystis* or *Entrophospora*. In *Glomus*, spores typically germinate either by a germ tube that penetrates through the subtending hypha or by germ tubes that emerge directly through spore wall (Hall, 1977; Walker and Rhodes, 1981; Miller and Walker, 1986; Nicolson and Gerdemann, 1968).

VAM fungi produce two types of mycelial systems, an internal mycelium within the epidermis and cortex of the host roots, and an external mycelium in the soil (Harley and Smith, 1983). In *Scutellospora*, septate hyphae are found within roots and external vesicles (auxiliary cells) are produced. In *Acaulospora* vesicles form intracellularly only, hyphae are non septate inter or intracellular (Abbott, 1982). Harley and Smith (1983) suggested that vesicles may perform the function of storage because lipids and glycolipids are the most abundant substances in them. Biermann and Linderman (1983) thought that intraradical vesicles in some species of VAM fungi act as propagules and contribute significantly to the infection of other roots.

Morton (1985) and Hall (1977) reached the conclusion that the morphological 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.

Abbott (1982) developed an identification key to ten species of VAM fungi that was based entirely on the morphological anatomy of hyphal development in VAM fungi. She used twenty characteristics, including hyphal diameter, mode of branching, vesicles, arbuscules, and staining reaction, in her key. Of all known species of VAM fungi, the spores of *Glomus tenue* (Green et al., 1976) are the smallest with average diameter 10-12 μm. While *Gigaspora gigantea* is the largest and ranges from 183-500 x 291-812 μm. The differences in colour can be due to pigmentation in the spore wall or in the spore content (Morton, 1988).
Spores in *Glomus* and *Sclerocystis* are formed terminally on one or more hyphae. It has been suggested that certain hyphal characteristics such as long infection units with ‘H’ connections between parallel strands of hyphae in *Glomus* (Abbott and Robson, 1979) pale staining of intraradical hyphae by trypan blue in *Acaulospora* (Bentivenga and Morton, 1994) constriction near branch points in hyphae of *Acaulospora* and *Entrophospora*, and irregularly coiled swollen hyphae with lateral projections or knobs in *Gigaspora* or *Scutellospora* may be utilized as diagnostic features to identify genera in mycorrhizal roots (Morton and Bentivenga, 1994). It is possible to differentiate among certain AMF using visual differences in morphology of fungal hyphae and vesicles within roots (Abbott and Gazey, 1994).

Traditionally, morphological characteristics of the spore walls of VAM fungi are used in species identification (Mosse and Bowen, 1968; Gerdemann and Trappe, 1974; Trappe, 1982). It became the most important morphological characteristic after Walker (1983) who suggested using standardized terminology and wall murographs. Spore wall characteristics have been universally accepted as more stable and reliable criteria than other spore features (Mehrotra, 1997). A spore wall is defined as the first individual structure to be formed, originating from the wall of sporogenous hypha and differentiating into phenotypically distinctive layers (Morton, 1995). Seven wall layer types, i.e. evanescent, laminated, membranous unit (Walker, 1983), expanding (Berch and Koske, 1986), coriaceous (Walker, 1986), amorphous (Morton, 1986) and germinal (Spain *et al.*, 1989) have been described so far. Differentiation of subcellular morphological characteristics in spores of *Gigaspora* (Bentivenga and Morton, 1994) and *Scutellospora* species (Morton, 1995) is used for identification.

Most *Glomus* species have a single subtending hypha on their spore (Morton, 1988). The colour of subtending hyphae in most species of *Glomus*, *Sclerocystis*, *Gigaspora*, and *Scutellospora* is similar or lighter than spore wall colour. Subtending hyphal shapes range from straight, recurved, cylindrical, flared, funnel-shaped, constricted or irregular. The opening between the subtending hypha and the spore content may remain open or may be closed by a plug, a septum, a spore wall thickening or spore inner wall.


Phylogenetic studies using DNA-sequence data suggest that the agarics are derived from wood rotting fungi (e.g. polypores), and two of the largest ectomycorrhizal groups, the Boletales and Russulales, are sister to most other agarics (Moncalvo et al., 2000). Phylogenetic studies based on SSU rDNA (18S) sequence data show that Geosiphon is a primitive glomalean fungus (Tehler et al., 2000; Schüßler et al., 2001). Geosiphon associations occur in swollen hyphae with an endosymbiotic interface similar to the arbuscule interface of VAM (Schüßler and Kluge, 2000).

On the basis of developmental patterns of morphological characters genus Gigaspora was redescribed by Bentivenga and Morton (1994). In Gigaspora, the auxiliary cells are echinulate with spines that are forked dichotomously Bentivenga and Morton (1994), whereas in Scutellospora, the projections on the surface of the auxiliary cells are highly variable in shape and size (Morton, 1995). The genus Glomites was erected by Taylor et al., (1995) to describe fossil fungi that closely resemble modern day Glomus species. Simon et al., (1993); Gehrig et al., (1996) and Redecker et al., (2000a) suggested on the basis of molecular studies that Glomus is a polyphyletic corporation of distantly related heredity, and some morphological characters previously used to define the genus.

coremioides. Schüßler et al., (2001) transferred AMF from the polyphyletic phylum Zygomycotina to newly erected monophyletic phylum Glomeromycota. They analysed AMF and the endocyctobiotic fungus Geosiphon pyriformis phylogenetically by their SSU (small subunit) rRNA gene sequences.

Ectomycorrhizal fungi include at least 6000 species, primarily of basidiomycetes with some ascomycetes and Zygomycetes (Molina et al., 1992; Castellano and Bougher, 1994). Recognition of fungi by mycorrhizal morphology (Agerer, 1995; Massicotte et al., 1999) lipid profiles (Olsson, 1999) or DNA-based methods (Gardes and Bruns, 1996; Jonsson et al., 1999) have shown that ectomycorrhizal roots often contain fungi that cannot be linked to epigeous fruiting bodies. These cryptic fungi may produce hypogeous sequestrate (truffle-like) (Bougher and Lebel, 2000) or resupinate (crusting) fruiting bodies (Erland and Taylor, 1999) or they may be sterile like the widespread fungus Cenococcum geophilum (LoBuglio et al., 1996; Shinohara et al., 1999) or fruit very infrequently.

The studies on sexual reproduction indicated that the Endogonaceae belong to the Mucorales (Bucholtz, 1912). Shortly thereafter Thaxter (1922) monographed all known Endogonaceous species. The family Endogonaceae now is considered to be in the class Zygomycetes (Benjamin, 1979). The genus *Endogone*, undoubtedly a member of the Zygomycetes produces characteristic zygospores by the fusion of two gametangia.

Early taxonomists thought that the large, globose zygospores, chlamydospores, or sporangia in the Endogonaceous fungi were asci, and they included the entire family in the Ascomycetes (Gerdemann and Trappe, 1974). The first comprehensive Linnaean classification was proposed by Gerdemann and Trappe (1974) who resurrected Glomus in the Endogonaceae and moved several species from *Endogone* to *Glomus*. They also described the genera *Acaulospora* and *Gigaspora* in 1974.

Examples of mycorrhizal fungi can be found in the class Zygomycetes, in which the fungal mycelium is aseptate (Alexopoulos and Mims, 1979). Ames and Schneider (1979) described the genus *Entrophospora*. Later Berch (1985) emended the genus *Acaulospora*, while Walker and Sanders (1986) transferred member species
of *Gigaspora* to another genus *Scutellospora*, based on the presence of sub cellular structures associated with germination.

Mosse and Bowen (1968) considered developmental stages of spores as major characteristics. Later, Gerdemann and Trappe (1974) separated the genera in the Endogonaceae based on spore morphological criteria, spore-bearing structures, and sporocarp morphology. Because of the abundant information on the Endogonaceae, Gerdemann and Trappe (1974) suggested a division of *Endogone*, the largest and the most heterogeneous genus in the family Endogonaceae, into four genera like *Endogone* (Link, 1809) which produces zygospores and does not form VAM fungal associations; *Glomus* (Tulasne and Tulasne, 1844) which produces sporocarpic or nonsporocarpic chlamydospores; *Gigaspora* (Gerdemann and Trappe, 1974) which is nonsporocarpic and produces azygospores; and *Modicella* (Kanouse, 1936) which produces thin-wall sporangia and was not known to form mycorrhizal associations.

On the basis of spore germination, spore wall structure, and morphology of auxiliary cells, the genus *Gigaspora* was split into two genera, *Gigaspora* and *Scutellospora*. *Glomus infrequens* was transferred into the new genus *Entrophospora*, as *E. infrequens*, because the fungal spores were unlike any other described species in the Endogonaceae (Ames and Schneider, 1979).

Gerdemann and Trappe (1974) described *Acaulospora* as a new genus in VAM fungi. This genus was characterized as nonsporocarpic, and produced spores borne singly and laterally on a hypha that terminates in a large thin-walled vesicle, and formed VAM fungal associations. Trappe and Schenck (1982) transferred the genus *Modicella* to the family Mortierellaceae in the order Mucorales of the Zygomycetes. The genus *Glaziella*, at one time, was included as a member of the Endogonaceae. However, Gibson *et al.*, (1986) transferred entire genus *Glaziella* to the Ascomycetes in a new family, Glaziellaceae, and a new order, Glaziellales.

Fungal anatomy in roots generally is not used in taxonomical descriptions to separate taxa below the generic level. Morton (1985) and Hall (1977) reached the conclusion that the morphological 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.
Trappe (1982) used morphological characteristics and spore germination as major characters in his synoptic key for VAM fungal classification. The genus *Endogone* is still classified according to its teleomorphic characteristics (sporocarps and zygospores). Tommerup and Sivasithamparam (1990) described zygospore formation in *Gigaspora*. Zygosporangia, zygospores or asexual sporangiospores are not reported in the genera *viz.* *Glomus*, *Sclerocystis*, *Acaulospora*, *Entrophospora*, and *Scutellospora*. Therefore these five genera cannot be placed in the Zygomycetes with certainty (Walker, 1987).

Among the newer approaches in VAM fungal classification is the use of isozyme analysis or immunological detection assays. Hepper *et al.*, (1986) used esterase (EST), glutamate oxaloacetate transaminase (GOT), and peptidase (PEP) as isozyme to identify *Glomus mosseae*, *G. caledonium*, and *G. fasciculatum* in *Zea mays* L. roots by the relative location of GOT and PEP activity for the first fungus and GOT activity for the third. Hepper *et al.*, (1986) concluded that there should be different mobilities for the host and fungal enzymes during electrophoresis when appropriate enzyme stains and running times are used. Wright *et al.*, (1987) reported the use of immunological methods for identification of *Glomus occulatum*. Simon *et al.*, (1992) were the first to report DNA sequences from VAM fungi. Simon *et al.*, (1993) adapted a new technique of making taxon-specific single-strand fluorescent probes of nuclear genes coding for small subunit rRNA. Redecker *et al.*, (2000b) carried out phylogenetic analysis of 18S ribosomal unit of *G. sinuosum* (*S. sinuosa*) and *S. coremioides*, and revealed that both species are the close relatives and fall within the *Glomus clade*.

Morton and Benny (1990) placed *Glomus*, *Sclerocystis*, *Acaulospora*, *Entrophospora*, *Gigaspora*, and *Scutellospora* in the new order Glomales, which includes two suborders: the Glomineae and the Gigasporineae. The Glomineae was separated into two families: the Glomaceae (Pirozynski and Dalpé, 1989) and Acaulosporaceae (Morton and Benny, 1990). The Gigasporineae (Morton and Benny, 1990) contained only one family, the Gigasporaceae with two genera, *Gigaspora* and *Scutellospora*. The monograph of VAM fungi includes the descriptions of 148 species (Schenck and Pérez, 1990). Our work is mainly carried out based on this monograph.
Two new families were erected by Morton and Redecker (2001) on the basis of some atypical morphological characters like striking immunological and fatty acid distance, and 18S rDNA sequence divergence. Two dimorphic sister species with similar ontogenetic sequences, *Acaulospora gerdemannii* and *Glomus gerdemannii* (*sensu lato*), together with *Acaulospora trappei* (*sensu lato*) were transferred to Archaeosporaceae. Two ancestral species previously classified in *Glomus*, *G. occultum*, and *G. brasilianum* (*sensu lato*) were grouped in a sister family, Paraglomaceae.

Schüßler et al., (2001) transferred AMF from the polyphyletic phylum Zygomycotina to newly erected monophyletic phylum Glomeromycota. They analysed AMF and the endocytobiotic fungus *Geosiphon pyriformis* phylogenetically by their SSU (small subunit) rRNA gene sequences. They reported that Glomeromycota probably diverged from the same common ancestor as the Ascomycota and Basidiomycota. They also erected new orders like Archaeosporales (Archaeosporaceae, Geosiphonaceae), Paraglomerales (Paraglomeraceae), Diversisporales (Acaulosporaceae, Diversisporaceae, Gigasporaceae), and Glomerales (*Glomus* – Group A, *Glomus* – Group B), with their respective families given in parenthesis.

Recent classification of ‘AM fungi’ updated December, 2010 by Schüßler and Walker (2010) states that class Glomeromycetes of phylum Glomeromycota consists of 4 orders, 11 families and 17 genera. The orders are Glomerales, Diversisporales, Paraglomerales and Archaeosporales.

First order Glomerales consists of two families Glomeraceae with four genera like *Glomus*, *Funneliformis*, *Rhizophagus*, *Sclerocystis* and second family Claroideoglomeraceae with single genus *ClaroideoGlomus*. Second order Diversisporales consists of five families like a) Gigasporaceae with three genera *Gigaspora*, *Scutellospora*, *Racocetra*, b) Acaulosporaceae with single genus *Acaulospora*, c) Entrophosporaceae with *Entrophospora* as single genus, d) Pacisporaceae with single *Pacispora* genus and e) Diversisporaceae consists of two genera *Diversispora* and *Otospora*. Third order Paraglomerales consist single family Paraglomeraceae with single *ParaGlomus* genus. Fourth order Archaeosporales consists of three families a) Geosiphonaceae with genus *Geosiphon* b) Ambisporaceae with genus *Ambispora* and c) Archaeosporaceae with genus *Archaeospora*. 

27
AMF (arbuscular mycorrhizal fungi) is widely distributed in agro-ecosystems (Smith and Read, 1997) forming symbiotic associations with the roots of plants. They play an important role in plant mineral nutrition and plant health (Barea et al., 2002; Ferrol et al., 2002; Giovannetti et al., 2002). These fungi have a wide range of application in sustainable low input agricultural systems (Schreiner and Bethlenfalvay, 1995). The use of AMF may contribute to reducing chemical fertilizer inputs and sustaining plant productivity in agriculture (McGonigle and Fitter, 1988). In natural soils in the presence of indigenous fungi, introduced isolates differ in their ability to stimulate plant growth (Medina et al., 1988).

Indian soils, was reported by Thaper et al., (1991) and Janardhanan et al., (1994). The marked difference observed in the composition of AMF can be attributed to the influence of agro-climatic conditions and edaphic factors. Redhead (1977) and Koske (1987) found that nutritional conditions of the soil played an important role in deciding the richness of the species and population density of AM fungi. Soil conditions such as pH, temperature, texture, and others also govern the diversity of AM fungi (Land and Schonbeck, 1991). Soil pH plays an important role in phosphorus availability in soil and uptake by plants (Wang et al., 1985). Soil pH, thus, has significant importance in VA mycorrhizal symbiosis and distribution of AMF (Janardhanan, et al., 1994).

The relation between moisture content and incidence of micro flora in root zones of crop plants was observed by Griffin (1963). High soil moisture or soil water potential reduces the infection of AMF by way of lowering oxygen tension of soils thereby hampering the chances of development of endophytes in the root region (Hayman, 1983).

Wind and animals are good vectors for spore dispersal (Friese, 1984). In arid ecosystems, wind might be the most important dispersal agent for VAM inoculums (Trappe, 1981; Warner et al., 1987). However, animals are the major vectors for VAM inoculum in mesic habitats (Allen, 1987). VAM fungal spores are usually more numerous and diverse in cultivated soils than under natural vegetation (Gerdemann, 1968; Mosse and Bowen, 1968; Hayman, 1978; Hayman and Stovold, 1979). Since VAM fungal spores are not easily dispersed from the point of sporulation, the upper
10 cm of soil is the best indicator of VAM fungal populations (Friese and Koske, 1991).

Mosses, the largest living group of bryophytes, are generally not mycorrhizal, but often contain endophytic hyphae of VAM fungi (Rabatin, 1980; Turnau et al., 1999). Liverworts and hornworts have VAM–like associations with glomalean fungi that form arbuscules in their thalli. Fine endophytes (glomalean fungi with very narrow hyphae forming VAM with arbuscules) are common in bryophytes, but other VAM fungi, such as *Glomus* species, are also present (Johnson, 1977; Turnau et al., 1999; Schüßler, 2000). Fine endophytes colonise roots of vascular plants in many habitats (Hall, 1977; Brundrett et al., 1999). Liverwort rhizoids are colonised by ericoid mycorrhizas in some ecosystems (Duckett and Read, 1995; Chambers et al., 1999; Read et al., 2000). Common liverwort is provided by the presence of arbuscules, the confinement of hyphae to specific tissues and the expression of different hyphal morphologies in different tissues (Ligrone and Lopes, 1989; Turnau et al., 1999). Several species of subterranean achlorophyllous bryophytes apparently have exploitative mycorrhizas (Leake, 1994; Read et al., 2000).

Taylor et al., (1995) and Phipps and Taylor (1996) provide the most detailed studies of mycorrhizas in vascular plants rhizome fossils. Schmid and Oberwinkler (1993) found subterranean gametophyte of a *Lycopodium* species with some characteristics of VAM. Gametophytes of another species of *Lycopodium* are similar, but have arbuscule-like structures in cells (Duckett and Ligrone, 1992). The hyphae within these gametophytes have similar ultra structural features to VAM fungi, but are extremely narrow, so are most likely to be a fine endophyte (Read et al., 2000). These gametophytes probably have exploitative VAM (Leake, 1994). Adult *Lycopodium* and *Selaginella* sporophytes have normal VAM associations (Gemma et al., 1992). *Isoetes* has VAM, even when growing as a submerged aquatic plant (Beck-Nielsen and Madsen, 2001). *Equisetum* sporophytes often have VAM with arbuscules, or can be devoid of mycorrhizas (Brundrett, 2002). Primitive ferns such as *Ophioglossum* have relatively thick roots which are consistently mycorrhizal (Boullard, 1979; Berch and Kendrick, 1982; Gemma et al., 1992; Unrug and Turnau, 1999; Zhao, 2000; Nair, 2001). Myco-heterotrophic VAM occurs in the subterranean gametophytes of *Ophioglossum* and *Botrychium* (Schmid and Oberwinkler, 1994; Read et al., 2000).
Adult plants of *Psilotum* are reported to have VAM with arbuscules in their rhizomes (Read et al., 2000).

Members of the Pinaceae have ectomycorrhiza and may have evolved from gymnosperms with VAM, or the Gnetales (Stewart and Rothwell, 1993). There are reports, such as the single ectomycorrhizal root of *Wollemia* observed by McGee et al., (1999) and the occasional ectomycorrhizal roots of *Juniperus* (Reinsvold and Reeves, 1986). Preserved imprints of roots of Podocarpaceae from the Lower Cretaceous have characteristic short swollen lateral roots called ‘mycorrhizal nodular roots’ (Cantrill and Douglas, 1988).

*Nymphaea* has VAM (Brundrett et al., 1999). The strongest evidence that VAM is the ancestral condition for angiosperms is provided its near-ubiquitous occurrence in them (Newman and Reddell, 1987; Trappe, 1987). Trappe (1987) compiled data for 6507 angiosperm species, of which 67% had VAM (including 12% considered to be facultative), 15% had another association type and 18% were non-mycorrhizal. Additional information for the UK flora (Harley and Harley, 1987), Hawaiian angiosperms 83% mycorrhizal Koske et al., (1992) and Australian plants (Brundrett, 1999) based primarily on plants from natural ecosystems have provided similar results. The mycorrhizal plants in ecosystems ranges from 100% (96% VAM, 4% ectomycorrhizal, < 1% non-mycorrhizal) in a Canadian deciduous forest (Brundrett and Kendrick, 1988) to 52% (35% VAM, 17% ectomycorrhiza, 45% non-mycorrhizal) in an Australian eucalypt forest (Brundrett and Abbott, 1995) or 40% VAM in a disturbed habitat (Barni and Siniscalco, 2000). The Fagales lineage includes the Betulaceae, Casuarinaceae, Juglandaceae, Myricaceae, Nothofagaceae, and Fagaceae (Chen et al., 1999), most of which have ectomycorrhizal roots. Fitter and Moyersoen (1996) suggest that ectomycorrhizal plants are concentrated in the rosids because there are many woody plants in this lineage.

Ericoid mycorrhizas occur in the Ericaceae and Epacridaceae (Kron et al., 1999). Phylogenetically, plants with arbutoid ectomycorrhizal (*Gaultheria, Arbutus, Pyrola*) are the sister group to the Ericaceae, with ericoid mycorrhizal plants as their monophyletic descendants (Cullings, 1996). *Clethra* (Clethraceae), which is basal to the remaining Ericales, has shown to have VAM (Kubota et al., 2001). Cullings
(1996) suggests that arbutoid mycorrhizas are intermediary between ectomycorrhizal and ericoid associations.

Families of predominantly non mycorrhizal plants include the Amaranthaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Commelinaceae, Cyperaceae, Juncaceae, and Polygonaceae (Tester et al., 1987; Brundrett, 1991).

The orders like Santalales and Lamiales have parasitic plants which are non mycorrhizal (Trappe, 1987; Lesica and Antibus, 1986), as do insectivorous plants in the Ericales and Lamiales (Lamont, 1982; Brundrett, 1999). Many epiphytes also are non-mycorrhizal, but others have VAM, orchid or ericoid associations (Janos, 1993; Gemma and Koske, 1995). Non mycorrhizal plants generally are most abundant in harsh plant habitats, such as extremely wet, saline or arid soils (Brundrett, 1991).

Greenhouse demonstrations of monocultural inoculum of VAM fungi showed general non-specificity with plant root systems (Gerdemann and Trappe, 1974; Harley and Smith, 1983). In contrast mycorrhizal host specificity is common in ectomycorrhizal fungi (Harley and Smith, 1983) and, to some extent, in mycorrhizal orchids (Warcup, 1951). However, in a recent study McGonigle and Fitter (1988) presented evidence that VAM fungal associations in the field can exhibit some degree of ecological specificity.

VAM fungi are found in tropical to alpine plants, and fungal development is strongly temperature dependent. The fungi are distributed over a wide range of soil habitats, including soil of arid regions of Pakistan (Khan, 1974) semiarid regions in the USA (Reeves et al., 1979) semi-arid regions of Australia (McGee, 1989) dry Sahel region of Nigeria (Redhead, 1977) to the wet soils of marshes (Dowding, 1959).

From the literature, Augè (2001) and Adivappar (2001) 80% of the mycorrhizal studies reporting plant growth during drought revealed the VAM plant to be larger than non mycorrhizal plants.

Occurrence of the AM infection and root colonization in several weed species was reported by Gupta and Ali (1996) and Manoharachary et al., (1988). There are very few reports on the AM–weed association in open-field environments (Bagyaraj and Varma, 1995).
Plant productivity in natural and managed ecosystems is often limited by nitrogen and phosphorus (Chapin et al., 1986), and interactions between the soil microflora and plants to increase nutrient availability become crucial (Richardson 2001, Biro and Koves-Pechy, 2000). AM fungi (AMF) influence soil carbon fluxes as well as nutrient dynamics of plants, soils, and the atmosphere (Treseder and Cross, 2006). AM fungi are key mediators between above-ground plant biomass and available soil nutrients (Read et al., 1992). Arbuscular mycorrhiza benefit the host plant by increasing uptake of phosphorus (P), zinc (Zn), copper (Cu), and nickel (Ni) and may increase resistance to soil-borne pathogens, insect herbivores, and drought (Smith and Read 1997; Wright, 2005). The primary benefit of forming arbuscular mycorrhiza for most plants occurs through the increased P uptake as phosphorus is one of the most limiting nutrients of plants. The necessity to form this symbiotic relationship with the AM fungi arises from the morphological and physiological characteristics of root systems combined with low levels of soil P and the immobility of P in the soil matrix (Wright, 2005). Many plant roots exhibit slow growth rates that make it extremely difficult to react to nutrient changes in the environment, and a large number of the roots also have insufficient branching, lateral roots, and root hairs to fulfil their P demands (Wright, 2005). Plants that form the symbiotic relationship with AM fungi depend on the AM hyphae as an extension of the roots to access a greater nutrient pool (Leyval et al., 2002; Wright, 2005). Furthermore, research has shown that mycorrhizal plants are more effective at using organic P than non mycorrhizal plants (Wright, 2005). The AMF mycelium receives 3-20% net photosynthate produced by host and 37-47% of carbon (C) delivered below ground (Treseder and Cross, 2006). This substantial allocation of C demonstrates how valuable this symbiosis is to plant nutrient uptake; otherwise the plant would not invest such a large percentage of net photosynthate to sustain the arbuscular mycorrhizal relationship. Most agricultural plants form arbuscular mycorrhizae and as a result there has been a greater effort made to understand how AM fungi can improve crop productivity and reduce fertilizer inputs (Smith and Read, 1997; Zak et al., 1998). Agro-management practices such as tillage, irrigation, herbicide and pesticide applications, and fertilizer applications can have substantial negative impacts on agricultural systems (Welbaum et al., 2004).
Application of nitrogen fertilizers reduced mycorrhizal colonization (Hayman, 1975; Alexander and Fairley, 1983; Menge, 1984). Sreenivasa and Bagyaraj (1989) showed that calcium ammonium nitrate applied at 80 ppm nitrogen levels produced maximum number of infective propagules of *Glomus fasciculatum* in association with Rhodes grass.

VAM fungi provide plants with phosphorus that enables mycorrhizal plants to grow better than non mycorrhizal plants when this element is limiting (Mosse, 1972; Williams *et al.*, 1974; Roncadori and Pokory, 1982; Koske and Polson, 1984; Backhaus *et al.*, 1986). Yield or biomass often is increased when plants form VAM fungal associations (Bolgiano *et al.*, 1983; Singh and Singh, 1986). VAM fungi improve growth in both nitrogen-fixing and non-nitrogen fixing plants. Singh and Singh (1986) inoculated lentil plants with *Glomus fasciculatum* and found that lentil showed mycorrhizal dependency for phosphorus uptake. *Glomus fasciculatum* increased *Rhizobium* nodulation, nitrogen fixation, lentil growth, and lentil yield. Citrus also appears to have a high level of mycorrhizal dependency, especially when rock phosphate is used as the phosphorus source. Mycorrhizal citrus plants show much better growth than non mycorrhizal citrus plants (Graham and Timmer, 1984).

Most observations of VA mycorrhizae are based on the use of Trypan blue (0.05%) to stain fungi in host roots (Phillips and Hayman, 1970). Kormanik *et al.*, (1980) described an acid fuschin technique in which clearing and staining of many plant root samples for observation can be accomplished. Brundrett *et al.*, (1984) developed another technique in which chlorazol black E allowed the detection of the developmental stages of VAM fungi in the host roots with more clarity than other techniques. Many species of *Gigaspora* and *Scutellospora* stain intensely with Trypan blue, regardless of the host species (Morton, 1988). *Acaulospora trappei* exhibits intermediate staining in Trypan blue (Abbott, 1982). *Glomus dimorphism*, *G. fecundisporum*, *G. leptoticum*, *G. maculosum*, *G. occultum*, *G. tortuosum*, *Acaulospora myriocarpa*, and *Entrophospora schenckii* are not stained or are weakly stained in Trypan blue (Morton, 1985). Ames *et al.*, (1982) developed a non-destructive approach to estimate fungal metabolic activities in structures within and outside the host roots.
Many micro-organisms including the AM fungi facilitate uptake of mineral nutrients from soil. Enhancement of growth and yield of the host plants has been reported due to AM inoculations (Freitas et al., 2004). Although specific fungus-plant associations with respect to drought tolerance are of great interest (Ruiz-Lozano and Azcon, 1996) the exact role of arbuscular mycorrhizal fungi (AFM) in drought resistance remains unclear (Augè et al., 1992). These fungi not only play a vital role in uptake kinetics of the host plants but also act as carrier and being symbiont, use to transport the nutrients into the host plant roots (Gupta and Baig, 2001). It is also worthwhile to note that the AM fungi help the host plants to grow under stressed environments (McMillen et al., 1998; Thaker and Jasrai, 2002; Gupta and Routaray, 2005). Water stress is the major edaphic limiting factor, which affects establishment and efficiency of mycorrhiza grown in association with various crops (AlKaraki et al., 2004). The effects of AM fungi on plant water status have been ascribed to the improved host nutrition (Graham and Syverten, 1987; Fitter, 1985); there are reports that drought resistance of AMF plants is somewhat independent of plant P nutrition status of plants (Augè et al., 1986; Bethlenfalvay et al., 1988; Khalvati et al., 2005). Vivas (2003) reported that the increased metabolically active fungal biomass in inoculated plants was independent of phosphorus levels and was not related to phosphorus uptake from the poor nutrients soil (Khalvati et al., 2005). Mycorrhizal benefits in plant mineral nutrition are being increasingly recognized in forestry, agriculture and plantation crops. Nehl et al., (1996, 1998) have shown that the mycorrhizal colonization, root browning and soil properties associated with a growth disorder of cotton in Australia and the slow arbuscular mycorrhizal colonization in field-grown cotton due to environmental conditions in the soil. Pattinson et al., (1997) studied the effect of fungicides Terrazole and Terraclor and the nematicide Fenamiphos on root colonization by Glomus mosseae and growth of cotton seedlings. Zak et al., (1998) studied Arbuscular-mycorrhizal colonization dynamics of cotton (Gossypium hirsutum L.) growing under several production systems on the Southern High Plains of Texas. McGee et al., (1999) have reviewed the relationship between density of Glomus mosseae propagules and the initiation and spread of arbuscular mycorrhizas in cotton roots. Feng et al., (2002a) have shown the uptake of nitrogen from indigenous soil pool by cotton plant inoculated with arbuscular mycorrhizal fungi. Hulugalle et al., (2004) studied soil properties, and cotton growth, yield and fibre quality in three cotton-based cropping systems. According to Sheng (2005) there
is growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of Bacillus edaphicus.

The global climate is rapidly changing; the productivity of the agriculture is also being seriously affected as a consequence of major shifts in the pattern of temperature and rainfall (Giorgi, 2005). It is anticipated that such changes will affect water availability to the plants, especially those native to the arid and semi-arid tropics.

Water availability is crucial for plant growth. Water is a major constituent (85 - 95%) of plant protoplasm. It provides turgidity to cells and tissues, which enables them to divide and differentiate. Moreover, it plays an important role in the translocation of salts and nutrients (Salisbury and Ross, 1992). Low rainfall and unavailability of sufficient irrigation water are major causes of limited water availability to crops in arid and semi-arid regions. The extreme spatial and temporal variability of water availability results in variable soil moisture. Thus water deficit cycles often occur during the life history of plants in such climatic zone (Belhassen and Monneveux, 1996; Passioura, 1996). It has also been reviewed that soil water scarcity, moisture deficit or drought may prove to be a critical constraint to agricultural productivity throughout the world (Fischer et al., 2001). Water unavailability can result crop losses and is considered as the second largest contributor to yield reduction after diseases (Khatri et al., 2004). Plants show numerous physiological, metabolic and molecular responses to water stress (Hoekstra et al., 2001; Chaves et al., 2002). All such changes are reflected by altered phenotype, where certain morphological traits can signify the abilities of plants to grow under water deficit conditions (Bray, 1997). Thus, the potential of the species for moisture deficit environment can be revealed by the variability of morphological expressions.

Salinity becomes a serious problem in arid and semi-arid regions and large area is a prone to salinity due to irrigation. Due to inadequate supply of fresh water, farmers are forced to use saline water to irrigate their crops. The interaction between salinity and nutrient uptake by plants is a complex task. The interaction is highly dependent upon the plant species, plant development age, the composition and level of salinity and the concentration of phosphorus in the substrate (Grattan and Grieve, 1999). Salinity affects yield quality and quantity of crops. Only yield-related characters are not important, as salinity affects almost every aspect of the physiology
and biochemistry of the plant. Therefore the enhancement of crop salt tolerance will require the combination of too many physiological traits (Flowers and Yeo, 1995; Cuartero and Fernández-Munoz, 1999) not only those directly influencing yield. Soil salinity adversely affects plant growth and development. Excess of salts in the soil leads to both osmotic and ionic stress (Munns, 2002; Benlloch-Gonzalez et al., 2005). Soil properties that inhibit or reduce plant survival and development include unfavorable pH, imbalance of essential ions and altered soil structure, factors which reduce aeration and water holding capacity (Bettenay, 1986).

The introduction of arbuscular mycorrhizal fungi to sites with saline soil may improve early plant tolerance and growth (Jain et al., 1989). AM symbiosis has frequently increased flexibility of host plants to salinity stress, perhaps with greater consistency than to drought stress. Growth in saline soils was increased by inoculation with Glomus species, with AM plants having increased phosphate and decreased Na concentrations in shoots compared to uninoculated controls (Pfeiffer and Bloss, 1987; Giri and Mukerji, 2004). Salt resistance was improved by AM colonization in maize (Feng et al., 2002), mung bean (Jindal et al., 1993) and clover (Ben Khaled et al., 2003) with the AM effect correlated with improved osmoregulation or proline accumulation. Arbuscular mycorrhizal fungi (AMF) are beneficial symbiotic fungi known to increase the nutrient uptake in plants, particularly phosphorus, sulphur, copper, and zinc (Abuzinadah and Read, 1989). AM colonization also improved NaCl resistance in tomato, with extent of improvement related to salt sensitivity of the cultivar (Al-Karaki, 2000; Al-Karaki et al., 2001). AM improvement of salt resistance has usually been associated with AM-induced increases in P acquisition and plant growth, although two of three AM fungi tested were able to protect cucumber plants from NaCl stress compared to similar sized non AM plants (Rosendahl and Rosendahl, 1991). AM fungi have been found to protect the roots from pathogen and nematodes (Bagyaraj, 1984). The hypothesis of our research was AM fungi play a significant role in protecting plants from salt stress. To prove this, we have chosen Bt cotton, one of the important cash crops from Maharashtra.

It has been accepted worldwide that AMF enhances plant mineral nutrition, especially phosphorus (P) (Mosse, 1973; Hayman, 1986). The majority of cultivated plants like Bt-Cotton (Gossypium hirsutum L.), is normally infected with the beneficial arbuscular mycorrhizal fungi. Moreover, mycorrhizal infection results in increase of growth in cotton (Hurlimann, 1974; Rich and Bird, 1974). It is found that
in the soil of low fertility where the symbiont increases efficiency of nutrient absorption by roots. Arbuscular mycorrhizae can explore greater amounts of soil and absorb more phosphorus and certain other minerals than non mycorrhizal roots (Hattingh et al., 1974; Ross and Harper, 1970). Nehl et al., (1998) have shown that the mycorrhizal colonization in root browning and soil properties associated with a growth disorder in Australian cotton. The environmental factors supports to the mycorrhizal colonization on roots in the soil (Ross and Harper, 1970; Schonbeck and Dehne, 1977).

Several studies indicate that mycorrhizal fungal root infections increase in mineral absorption which influence diseases caused by soil borne fungi. Mycorrhizal roots of a cultivar of soybean susceptible to Phytophthora were more susceptible than non mycorrhizal roots to P. megasperma (Ross, 1972). In contrast, VAM fungi caused to decrease in production and germination of chlamydomes of Thielaviopsis basicola (Baltruschat and Schonbeck, 1972) and increased resistance of tobacco to disease caused by T. basicola (Baltruschat and Schonbeck, 1975). Non mycorrhizal cotton roots were more severely damaged than mycorrhizal roots by T. basicola (Schonbeck and Dehne, 1977). The only one report on the influence of VAM on a vascular wilt of tomato caused by Fusarium oxysporum f. sp. lycopersici was reduced when plants were pre-infected with the mycorrhizal fungus Glomus mosseae (Dehne and Schonbeck, 1975).

The data compiled from the review of literature has given many aspects to study AM fungi associated with Bt cotton. With the help of review of literature different methods are established after studying and comparing the techniques to study taxonomy of AM fungi, effect of AM fungi on water stress tolerance, salt stress tolerance, growth and productivity in Bt cotton, interaction of AM fungi with Verticillium wilt. These methods are described in next chapter.