Chapter 2
REVIEW OF LITERATURE
Arbuscular mycorrhizas may have been described as early as 1842 (Nageli 1842), but most of Nageli’s drawings only remotely resemble the arbuscular mycorrhiza. Trappe and Berch (1985) and Rayner (1926–1927) cite other 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 already 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, for example, that it is entirely 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 years 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. Light and electron microscopical studies of arbuscular mycorrhizas were facilitated by the founding in 1950 of the Centro di Studio sulla Micologia del Terreno by Peyronel in Torino, Italy (Bonfante 1991). There, Scanderini 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.

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). However, Janse (1887), Peyronel (1940) and Bereraz (1960) have already reported use of KOH as clearing agent in their studied. 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 and references therein). 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, 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 identities that at one point the possibility were 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

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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. Janse failed, as did Gallaud (Rayner 1926–1927), Peyronel (Harley 1991) and Jones (1924). 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 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, 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 an *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 nonsterile sporocarps of a fungus initially named *Endogone mosseae* in her honor (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 (1955a) also 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. The experiments of Gerdemann and Mosse thus well established the absence of a strict host-specificity by at least some arbuscular mycorrhizal fungi, and together provided evidence that arbuscular mycorrhizas could be caused by more than one species of fungus. Gerdemann (1955a) was careful to note that the mycorrhiza from his "type B" spores was arbuscular and that no vesicles were produced, which distinguished his fungus from the one used by Mosse. It thus became clear that there were at least two patterns of symbiotic development by arbuscular mycorrhizal fungi.
Frank (1885) gave the name "mycorrhiza" to the peculiar association between tree roots and ectomycorrhizal fungi. Thorough discussion of the derivation of the word "mycorrhiza", including the incorporation of the second 'r' is given by Kelley (1931, 1950). In another publication, Frank (1887) recognized a distinction between ectotrophic and endotrophic mycorrhizas, which included at the time only ericaceous and orchid mycorrhizas. The name for the arbuscular mycorrhizal symbiosis has changed through the years. The symbiosis was once frequently called "phycomycetous endomycorrhiza" to distinguish it from the endomycorrhizal symbioses formed between members of the Ericaceae or Orchidaceae and higher fungi. The name "Phycomycete", however, no longer carries any systematic significance. As previously mentioned, Janse (1897) called the intramatrical spores "Vesicules" and Gallaud (1905) called the other commonly observed intracellular structures "arbuscules". Thus the name "vesicular-arbuscular mycorrhiza" was established and persisted until recently. The recognition that not all fungi formed vesicles led to the proposal that this symbiosis should be renamed arbuscular mycorrhiza. This change is now widely accepted, but in some of these associations the fungi may not even produce proper arbuscules (Smith and Smith 1997). Moreover, one must agree that some hosts of arbuscular mycorrhizal fungi do not house the fungi in true roots at all, and therefore that the name "mycorrhiza" is not correctly used in those cases (Lohman 1927; Kelley 1931). If we continue with the line of reasoning that dropped the "vesicular" from the "vesicular-arbuscular mycorrhiza", we must also drop the "arbuscular" and, if we wish to be more inclusive of associations involving these fungi, we must also drop the "- rhiza". We would then be left only with "myco-" and that is useless. Perhaps "phycomycetous endomycorrhiza" was not such a bad choice after all. We are having fun here, of course, but it is interesting to note the continual problem we have had with names. Although it is no laughing matter, one might be amused to count the times we have questioned what should even be considered a mycorrhiza in the first place (Boullard 1982; Allen 1996; Trappe 1996; Jones and Smith 2003; Massicotte and Peterson 2003).
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. Thus, as recently as 25 years ago the nomenclature of the arbuscular mycorrhiza fungi had not been firmly established.

The history of the naming of mycorrhizal fungi is certainly an interesting one. Link (1809, cited in Gerdemann 1971), 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. The Tulasne brothers considered *Glomus* to be closely related to *Endogone*. 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. He regarded this as a disease and named the fungus *Rhizophagus populinus* (Dangeard 1900), provisionally placing it within the chytridiales. In 1922 Thaxter revised the endogonaceae, placing the *Glomus* of Tulasne and Tulasne into *Endogone*. He considered *Endogone* to contain both zygosporic (notably *Endogone lactiflua*) and chlamydosporic species, observing that at least one species apparently produced both kinds of spores. In 1939 Butler, in reviewing the identity of arbuscular mycorrhizal fungi, classified them as probable imperfect members of the endogonaceae. He nevertheless accepted the name *Rhizophagus* for such fungi because of the earlier naming by Dangeard.

The extraction of spores from soil is necessary for their classification. Routine extraction from soil was made possible by wet sieving and decanting, a method commonly used to extract nematodes from soil and adapted to arbuscular mycorrhizal fungi by Gerdemann.
(Gerdemann 1955a; Gerdemann and Nicolson 1963). Mosse (1953), Gerdemann (1955a, 1961, 1965) and Gerdemann and Nicolson (1962, 1963) added more species to Peyronel's (1924, 1937) existing list of *Endogone*, whose spores could produce typical arbuscular mycorrhizas. Gilmore (1968) further added to the list by describing six spore types, E2–E7, found in pot cultures. All these "species" based on spore type seem to have little in common except that they produced aseptate multinuclear hyphae, extramatrical spores, intracellular arbuscules or hyphal coils, and could not be cultured. At this point it seemed time to attempt some classification or method of recognition of all arbuscular mycorrhizal spore types. Nicolson and Gerdemann, both plant pathologists by training, decided on the classical system with Latin names. Mosse (a plant anatomist) and Bowen (an ecologist) attempted a more descriptive system based mainly on spore wall structure and color, and cytoplasmic characteristics (Mosse and Bowen 1968). Nicolson and Gerdemann (1968) divided the fungi into two groups of *Endogone*, one forming extramatrical azygospores/zygospores arising from the tip of a swollen hyphal suspensor but producing no intramatrical vesicles, corresponding to the bulbous vacuolate and bulbous reticulate types of Mosse and Bowen (1968), and the other forming extramatrical chlamydospores and intramatrical vesicles corresponding to the yellow vacuolated and red brown laminate spores of Mosse and Bowen (1968). There was thus some correspondence between the two attempts at classification. Because spores possessed so few distinguishing features, which were frequently affected by age and environment, the naming of new species became quite a popular pursuit, but the E3 type of Gilmore, which is quite common in nature, did not and has not since found a home anywhere.

In the early 1970s it became clear to Gerdemann and Trappe (Gerdemann and Trappe, 1974) that *Endogone*, which now contained a wide variety of species, needed further revision. They split the old *Endogone sensulato* into seven genera including *Endogone*, *Modicella*, *Giaziella* (nonmycorrhizal genera), and four mycorrhizal genera including *Glomus* (which they resurrected, and which had also previously been
referred to as *Rhizophagus*), a previously described mycorrhizal genus, *Sclerocystis*, and two new genera *Gigaspora* and *Acaulospora*, which corresponded to the honey-colored sessile spores of Mosse and Bowen (1968). These were all placed in the endogonaceae, endogonales, zygomycetes.

Trappe and Schenck (1982) recognized another mycorrhizal genus, *Entrophospora*. In 1987, Walker also recognized five arbuscular mycorrhizal fungal genera, having dropped *Sclerocystis* and added *Scutellospora*. In 1990, Morton and Benny placed the five genera of Walker (1987) into three families (Glomaceae, Acaulosporaceae, Gigasporaceae) and two suborders (the Glomineae and the Gigasporineae), both of which were then placed in a new order, the Glomales. Later, Morton and Benny (2001) recognized two other families, the archaeosporaceae and paraglomaceae, with two new genera, *Archaeospora* and *Paraglomus*.

In 2001 Schubler *et al.* used molecular data to establish the relationships among arbuscular mycorrhizal fungi and between arbuscular mycorrhizal fungi and other fungi. The group of arbuscular mycorrhizal fungi was elevated to the level of phylum (glomeromycota), which was shown to be as distinct from other fungi as the ascomycota are from the basidiomycota. Little did the early researchers know that they were studying an entirely new phylum of fungi. The zygomycota were shown to be polyphyletic, and *Endogone* did not group near the glomeromycota nor did it group with the mucorales. *Geosyphon pyriforme* was added to the glomeromycota, which may have far reaching effects on our understanding of the arbuscular mycorrhizal symbiosis.

Walker and Schubler (2004) proposed the modern taxonomy of Phylum glomeromycota based on SSU r-DNA sequence. They divided phylum glomeromycota into the four orders i.e. archaeosporales, diversisporales, glomales and paraglomales. The order archaeosporales is divided into 2 families archaeosporaceae (*Archaeospora* 3 species) and geosiphonaceae (*Geosiphon* 1 species). The order diversisporales, are divided into 4 families i.e. acaulosporaceae (*Acaulospora* 32 species,
Entrophospora 5 species), diversisporaceae (Diversispora 1 species, species from Glomus group C, 1 species) and gigasporaceae (Gigaspora 10 species, Scutellospora 33 species) and pacisporales (Pacispora 7 species). The order glomales having single family glomaceae (Glomus 103 species). The little one order paraglomales having one genus Paraglomus with two species.

Research on the potential value of arbuscular mycorrhizal fungi in agriculture and land reclamation followed from the discoveries in the 1950s, 1960s and 1970s that they could substantially increase P uptake and plant growth under certain circumstances. However, the increasing number of observations that such fungi already exist in most agricultural soils led some to conclude that there would be little value in inoculation (Menge, 1985). Khan (1972) may have been among the first to demonstrate that such a practice could be beneficial in some circumstances, but it would frequently prove to be uneconomic because of the large cost of inoculum production relative to the cost of phosphate fertilizer (Menge, 1985). However, the practicality of inoculating soils that are inherently low in inoculum potential such as sterile citrus nursery beds (Menge et al., 1977), sterile potting media, or soils that are highly disturbed may be greater. For example, the revegetation of disturbed lands, and the course of plant succession in such environments may be strongly influenced by inoculation with mycorrhizal fungi. Much of the pertinent literature on use of arbuscular mycorrhizal fungi in land reclamation was summarized in a publication edited by Williams and Allen (1984). The poem "Them Spore Pickers", written by Allen and included in that publication, is a real gem.

Potting "soils" used in the greenhouse is typically formulated from mixtures of materials such as peat moss, perlite and vermiculite, and thus lack mycorrhizal fungi. Inocula based on peat moss have been developed (Parent, 1990), and these are capable of enhancing plant growth under some conditions (Ponton et al., 1990a, 1990b). However, it is not clear that the typical benefits of mycorrhizal fungi in increased phosphate uptake will always occur in potting media with low
P adsorption (Biermann and Linderman, 1983). Nevertheless, non-nutritional effects of mycorrhizal fungi, such as those on root branching (Berta et al., 1990, 1991), ethylene production (McArthur and Knowles, 1992; Besmer and Koide, 1999) or protection from pathogens may still be important.

Jones (1924) found that a diversity of soils across the United States supported arbuscular mycorrhizal plants. Lohman (1927), Peups (1950), Porter et al. (1987) studied the effects of soil pH on mycorrhization. Furlan and Fortin (1973), Hayman (1974) studied the effect of the temperature and Reid and Bowen (1979) on the symbiosis, but Jones (1924) was probably the first to investigate these relationships. The effects of freezing or drying on survival of the fungi do not appear to have been examined until relatively recently (Jasper et al., 1989; Addy et al., 1994; Kabir et al., 1997; Klironomos et al., 2001). The nature of spore dormancy and the environmental factors that overcome it have been investigated Mosse (1959a), Siqueira et al. (1985). Schwab and Reeves (1981), Koide and Mooney (1987), have that mycorrhiza inoculum potential varies with soil depth but this had already been noted earlier by Jones (1924).

The early observations that plant species differed in their response to mycorrhizal fungi (Lohman, 1927; Baylis, 1970, 1972b) and that some plant species were nonmycorrhizal, led to the hypothesis that the fungi could help to structure natural plant communities. Indeed, mycorrhizal fungi may influence the course of plant succession (Nicolson, 1960; Janos, 1980) and the relative competitive abilities of host plants (Crush, 1974; Fitter, 1977; Hall, 1978).

Although there is no evidence of strict host-fungus specificity with arbuscular mycorrhizas (Helgason et al., 2002), the composition of the mycorrhizal fungal community has the potential to both influence (van der Heijden et al., 1998) and is influenced by (Hetrick and Bloom, 1983; Anderson and Liberta, 1985; Bever et al., 1996) plant community composition. These interactions clearly have relevance to
agro-ecosystems, particularly where crop rotations or intercropping are involved.

MYCORRHIZAL RESEARCH IN INDIA

In India Bakshi (1974) was the first to publish an account of 14 spore types: Glomus macrocarpum, Glomus macrocarpum Tul. and Tul. var. geosporum, Glomus mosseae, Glomus sp., Sclerocystis coremioides Berk. and Broom, Sclerocystis sp. Gigaspora calospora, Acaulospora sp., Endogone gigantea Nicol. and Gerd., Endogone microcarpum, Endogone 1, Endogone 2, Endogone 3. Gerdemann and Bakhshi (1976) reported two new species viz. Glomus multicaule and Sclerocystis sinuosa. Bhattacharjee and Mukerji (1982) described the species Glomus reticulatum from the soil of Bangalore. Bhattacharjee et al. (1982) reported the structure and hyperparasitism for Gigaspora candida while Bhattacharjee and Mukerji (1983) described the ultra-structure of Sclerocystis coremioides sporocorp. Mukerji et al., (1983) reported two species of Glomus, viz. Glomus multistentensum and Glomus delhiense both from soils of Delhi. Till this date, 102 AM species have been reported from India.

The occurrence of AM fungi in a natural forest stand was recorded in a natural forest stand was recorded in the Old Delhi Ridge, Saraswati Range of Haryana (Thaper and Uniyal 1996). The occurrence of AMF in various part of Indian forest was recorded by Manoharachary and Rao (1991), Ganesan et al. (1991), Raman and Nagarajan (1995), Kulkarni et al. (1997), Beena et al. (2000), Khade and Radrigues (2003).

Sengupta and Chaudhari (1989, 1990) and Selvaraj and Subramanian (1991) studied the occurrence of AM fungi in arid and semiarid regions were studied in Tamilnadu (Parthipon et al., 1991), deserts (Neeraj and Verma 1991) arid zones of Rajasthan (Mohan and Verma 1995) and semiarid grasslands of Mauthamalai hills (Western Ghats in Peninsula India) Muthukumar and Udayan (1995).

Nalini et al. (1987) 1st time reported the AMF diversity in agricultural fields. After that Kehri et al. (1987); Dalal and Hippalgaonkar


Rice is probably the oldest of food crops. It has been in cultivated from very ancient times in China, India and probably Africa, rice forms the principle food of nearly half of the world population. India is the largest rice growing country in the world.

Rice being the major agricultural crop of our country occupying about 37% of the total area under cereals. It covers a total area of 42.31 million hectares, the world's largest rice area. The total yield of this vast area, although poor some years back, has now risen to 73.66 million tonnes a year with the cultivation of some special, prolific varieties. However, Asia as a whole, accounts for 90% of the world's total rice production. Rice is the staple food in our country and tropical Asia, and feeds over 60% of the world's population. Its pericarp and embryo contain 70-80% starch, 7% protein, 1.5% oils, some vitamins (mostly A, B and C) and some essential minerals.

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Two or three cropping are practiced in our country according to the conditions of the soil and the climate. In our country rice fields generally remain under-manured. Common seasonal varieties are AUS or summer rice, AMAN or winter rice and BORO rice, which is grown between the two seasons, with many sub-varieties like Taichung native 1, Tainan 3, Kalimpong 1, Jaya, Padma, IR-8, IR-24 and IR-36, all high yielding and early-maturing may be cultivated on the same land, BORO paddy in April, AUS paddy in July and AMAN paddy in October or even earlier. ADT 27, now under cultivation in parts of Chennai, yields about 4,257 kg/hac per year. Further two new fine, dwarf, high-yielding varieties, IET 1919 and IET 1039 have shown great promise in several parts of Tamil Nadu.

In our country productivity in rice is only 1.75 tonnes/hac, while that of Egypt is 5.6 tonnes/hac, Korea 6 tonnes/hac, Japan 5.8 tonnes/hac and Australia 5.5 tonnes/hac. Recently Zhang and Wang (2005) reported that China is producing rice 5.4 tonnes/hac.