Chapter 1

General introduction

Mycorrhizae are symbiotic associations between the roots of plant species and fungi. The term “mycorrhiza”, which literally means “fungus roots”, was first applied to fungus tree association described in 1885 by German forest pathologist A.B. Frank in forest tree such as pine. Since then, it has been established that the vast majority of land plants form symbiotic association with fungi; an estimated 95% of all plant species belong to the genera that characteristically form mycorrhiza. Endomycorrhizae are mainly formed by two types of fungi i.e. with septate hyphae and non-septate hyphae. Septate hyphae type fungi are common in orchidaceous and ericaceous group of plants. Non septate hyphae fungi commonly called vesicular arbuscular mycorrhiza. VA mycorrhiza are common in Bryophytes, Pteridophytes, Gymnosperms and Angiosperm plants. VA endophytes develop vesicles and arbuscules. They also produce zygospores, chlamydospores and sporocarps. The AM fungi are placed in division Glomeromycota under it’s recent classification (Schubler et al., 2001). The AM is grouped into three families Glomaceae, Acaulosporaceae and Gigasporaceae having all total six genera viz, Glomus, Sclerocystis, Acaulospora, Entrophosphora, Gigaspora and Scutellospora.

Tree species of forest and woodlands are either ectomycorrhizal or arbuscular mycorrhizal (AM), while herbaceous plants and shrubs in shrublands and grasslands can be ericoid, orchid or AM (Smith and Reed 1997). AM associations are the outcome of 450 million years of co-evolution, which has led
to adaptations of symbiotic development and function both in plants and fungi. The main physiological basis for mutualism is bi-directional nutrient transfer (Smith and Smith 1990). Plants supply the AM fungi with sugars and the fungi enhance the ability of the plants to uptake scarce and immobile nutrients, particularly P. Mycorrhizal fungi can also benefit plants by drought tolerance, hormone production, heavy metal tolerance (Zhang et al 2005) and potentially protecting roots from plant pathogens (Graham 2001).

Hyphae of mycorrhizal fungi have the potential to greatly increase the absorbing surface area of root. If the mycorrhiza is to be effective in nutrient uptake, the hyphae must be distributed beyond the nutrient depletion zone that develops around the root. A nutrient depletion zone develops when nutrients are removed from the soil solution more rapidly than they can be replaced by diffusion. For a poorly mobile ion such as phosphate, a sharp and narrow depletion zone develops close to the roots. Hyphae can readily bridge the depletion zone and grow into soil with adequate supply of phosphorus.

In recent years, the importance of AM fungal diversity of plant diversity, productivity and ecosystem processes has been recognized. Several recent studies have demonstrated that AM fungi are common and ecologically important in tropical ecosystem, and that co-occurring plant species vary considerably in their germination, growth and flowering response to mycorrhizal colonization along a continuum from highly responsive, obligate mycotrophic species to facultatively mycotrophic and non-responsive species (Johnson et al 1997).
Evidences exist that mycorrhizal plants are capable of resisting the parasite invasion and minimize the losses caused by soil borne phytopathogens. Biocontrol of Fusarium in wilt of cotton, jute, tomato and Macrophomina root rot of cowpea was achieved by the application of VAM fungi (Caron et al. 1986; Ramaraj et al. 1988). Narayan Reddy et al. (1987) found significant reduction in pathogenic fungal population from mycorrhizal roots. However, Phytophthora root rot was increased by VA mycorrhiza in soyabean (Ross 1972) and avocado (Davis et al. 1978). Roots colonized by VAM harbour more actinomycetes antagonistic to root pathogens. The root pathogen Thileviopsis basicola is known to be inhibited by VAM fungi. Mycorrhizal plants are known to have an elevated level of phenols in the host cells compared to non-mycorrhizal plants which are an indicator of increased resistance to fungal hydrolytic enzymes (Dehne 1982; Krishna and Bagyaraj 1984). Cells containing arbuscules and intracellular hyphae have higher phenol content compared to the cortical cells devoid of the fungus (Krishna and Bagyaraj 1984). Mycorrhizal infection in plants stimulates biosynthesis of phytoalexins and their concentration has been found related to the extended colonization by fungi.

The beneficial effect on plant growth and yields following inoculation with VAM have been generally attributed to improved mineral nutrition (Smith and Daft 1977), mobilization of nutrients through greater soil exploration (Lambert et al. 1979; Abbott and Robson 1984; Cooper 1984), production of ectoenzymes (Dodd et al. 1987; Tarafdar and Classess 1988), protection of host roots against infection by pathogens (Dehne 1982; Thompson and Wildermuth 1988; Boyetchko and Tiwari 1988; Boyetchko and Tiwari 1990) improved water
relation, better tolerance to stress like salinity, heavy metal pollution and protection against transplantation shock, etc. (Dodd and Thompson 1994).

Various mechanisms proposed to account for the enhancement nutrient uptake includes physical exploration of soil, increased translocation of ‘P’ into plants through arbuscules and modification of the root environment (Hayman 1983). Among the nutrients the focus has been made on phosphorous because improved growth by VAM infection is most often correlated with high ‘P’ uptake by the plants (Bolan 1991). Although, it was reported that the mycorrhizal and non mycorrhizal plants appeared to use the same level ‘P’ sources (Mosse 1977; Gianinazzi-Pearson and Gianinazzi 1976). Jayachandran et al. (1992) had demonstrated that mycorrhizal plants can derive ‘P’ from organic sources which are not readily available to non-mycorrhizal plants. VAM fungal phosphatases are able to mineralize organic ‘P’ sources (Jayachandran et al. 1992; Tarafelan and Marschner 1994). The role of phosphatases in the transfer of ‘P’ to the host and their relatedness to arbuscules functioning was suggested by Gianinazzi-Pearson and Gianinazzi (1976, 1978). Alkaline phosphatase activity is related in PO₄⁻ metabolism of fungus as it is present within the fungal vacuoles where polyphosphate granules were observed. Dodd et al. (1987) reported that root and rhizosphere levels of phosphatase activity were higher in plant infected with *Glomus geosporus* and *G. mosseae* compared to control plants. Rubbio et al. (1990) reported that acid phosphatase activity of four mycorrhizal spring wheat cultivars was much higher than uninoculated control.

Soil pH plays a crucial role in uptaking plant nutrients by AM fungi, which influences the mobilization and availability of various essential and
functional elements in soil (Haynes, 1990; Marschner, 1995; Ingrid et al., 2002). Effect of soil pH on mycorrhizal fungi was studied by various workers who reported that intensity of infection and number of spores in the soil are affected by soil pH (Sheikh et al., 1975), and concluded that AM fungi has the highest efficiency in lower soil pH. Similarly, researchers found that application of Farm Yard Manure/ organic matter helps in efficiency of AM fungi by enhancing spore production (Johnsen and Mc Graw, 1988; Douds et al., 1997), extra radical proliferation of hyphae (St John et al., 1983; Joner and Jackobsen, 1995) and improve colonization of roots (Muthukumar and Udaiyian, 2000).

AM fungi are generally found in Phosphorus deficit soil (Smith and Read, 1997) where soil P remain in soil by inorganic fixation and formation of organic complexes (Schachtman et al. 1998; Abel et al. 2002). Under such condition, Mycorrhizal fungi lower the rhizospheric pH due to selective uptake of NH₄⁺ and release of H⁺ ions. Decreased soil pH increases the solubility of phosphorous precipitates (Hamel, 2004). Therefore, on application of higher dose of commercial P fertilizer in soil decreases the availability of AM fungi.

*Piper mullesua* D. Don. (syn *P. brachystachyum* Wall ex Hook. f), commonly known as Pipli, Pahari peepal, is indigenous to Arunachal Pradesh (India) and widely distributed in this the Eastern Himalayan region at an altitude of about 600m to 1500m. It is an important medicinal plant belonging to the family *Piperaceae*. Male and female flowers are found in separate spikes of the plant. Male spikes are 3-6 cm long, erect, slender and cylindrical. Female spikes are globose, oblong erect. Roots and fruiting spikes are used in treating diarrhea, indigestion, jaundice, urticacia, abdominal disorder, harseness of voice, asthma,
cough, piles, malaria fever, vomiting, wheezing, chest congestion, throat infection, worms and sinusitis. *Piper mullesua* is also considered as a rejuvenating plant. Myristicin, a 1,3-benzodioxole has been extracted from the hexane fraction of alcohol extract of fruit bearing inflorescence of *Piper mullesua* which has insecticidal properties (Srivastva *et al.* 2001).

For the commercial purpose, fruits of *Piper mullesua* are collected from wild habitat. Such haphazard and restless collection is resulting into loss of the plant from wild habitat. This depletion can be checked only by commercial cultivation, however high mortality of the plant in fields and their low yield are the problem associated with commercial cultivation. Application of efficient mycorrhizal fungi at seedling stage to piper plants may help in preventing mortality and enhancement of growth. A perusal of literature shows that there is no study on the role of mycorrhizal association with *Piper mullesua*.

Present study was undertaken on the contribution of arbuscular mycorrhizal symbiosis to the P. mullesua with the following objectives:

1. Isolation, characterization and culture preparation of AM fungi.
2. To evaluate the symbiotic efficiency of different AM fungi with *Piper (Piper mullesua)* plants at different pH, phosphorus and organic manure levels.
3. To study the performance of *P. mullesua* plants with efficient mycorrhizal fungi in sterilized and unsterilized soil as well as under field conditions