Introduction

Mycorrhizal fungi exist as a symbiotic association between a fungus and a root of a plant, in which the roots provide nutrients for the survival of the fungus and so they co-exist in nature (Brundrett, 2002). Mycorrhizal fungi are responsible for pivotal roles in both microbiological and ecological processes involving enhancement of soil fertility, decomposition, biogeochemical cycling of minerals and organic matter contributing plant health and nutrition (Haystead et al., 1988; Smith and Read 2008). Arbuscular mycorrhizal fungi (AMF) act as a significant agent in nutrient recycling (Haystead et al., 1988; Smith and Read 2008) which promotes the plant’s access to available water resource (Gavito and Varela, 1995), formation of soil aggregates (Hamel et al., 1997) and promote plant biodiversity (Van der Heijden et al., 1998b). Apart from the above factors, the AMF also involved in the protection of the plants against various pests and diseases as reviewed by Duchesne et al. (1989), Kapoor and Mukerji (1998), Kegler and Gottwald (1998) and Becker et al. (1999).

Mycorrhizal fungi are ubiquitous in nature accounting for 5-36% of the total biomass in soil and 9–55% of the biomass of soil microorganisms (Olsson et al., 1999). Moreover, these fungi have prominent roles in plant growth promotion (Smith and Read, 1997). Banana and plantain cultivars are naturally colonized by arbuscular mycorrhiza fungi. AMF contributes as a reliable option for chemical fertilizers and biopesticides leading to sustainable production (Bethlenfalvay and Schuepp, 1994; Jeffries and Barea, 1994; Hooker and Black, 1995).

The potential challenges in mycorrhizal studies are attributed to their biodiversity and phylogeny owing to their rapid and unambiguous identification. Until the recent past, the taxonomy of AMF largely depends on the morphology of the spores. The hyphal attachment and mode of formation of these spores are utilized in classification of families and genera, while sub structures of the walls are the prime resource in species identification. The formation of spore characteristics depends on environmental stimulus rendering their arrangement unpredictable (Schenck and Perez, 1990). Under these conditions, the molecular characterization would provide a precise mode of identification even in the absence of spores. In pursuit for molecular
identification strategies, PCR-based techniques pose a consistent tool in robust
diagnosis and detection of plant pathogenic fungi (Waalwijk et al., 2004; Lievens et al.,
2005). Nevertheless, the restriction digestion of PCR products from 18S rRNA genes
have been increasingly used in phylogenetic classification and biodiversity studies
(Simon et al., 1993b). The diversified nature of AMF has been assessed in number of
studies for natural communities (Clapp et al., 1995; Claassen et al., 1996), between
different species (Redecker et al., 1997), among individual isolates (Lloyd-Macglip et
al., 1996) and specifically within one spore (Sanders et al., 1995). Interestingly, it was
found that, the sequence variation in single spores are not of much significance than
isolates of one species from different origins. Ecological limitations in morphology-
based identification of the Glomales are of limited use in ecological settings as spore
production depends on physiological parameters and not because of root colonization
(Merry weather and Fitter, 1998). Therefore, an importance for molecular identification
of AMF based on ribosomal DNA sequences is very much essential (Simon et al.,

AM fungal diversity varies the rhizospheric potential thereby altering the soil
fertility on a large scale. Therefore, the AMF diversity analysis remains an important
factor, as the AM fungi are of strict biotrophic nature and also due to the difficulties in
obtaining sufficient fungal material and lack of knowledge of the reproductive system
and mutation rate. The parameters like presence of high numbers of nuclei in AMF
spores (Biancotto and Bonfante, 1993), intrasporal variations in Gigaspora margarita
(Lanfranco et al., 1999) showed that identification of AMF was still a difficult
protocol. The AMF in conferring resistance against pest and disease particularly
Fusarium wilt disease in banana was the primary objective. Understanding the diversity
and accurate identification of AM fungi by molecular methods will pave way for the
banana specific AMF which in turn will be useful for increasing the banana health
against various infectious diseases.

Banana plantations are prone to various fungal infections, among which the
Fusarium wilt caused by Fusarium oxysporum f.sp. cubense (Foc) accounts for fatal
damage worldwide (Moore et al., 2001). In India also, the banana production affected
by Fusarium wilt disease has been extensively reviewed (Mustaffa and Thangavelu,
enter the plants through the roots thereby blocking the vascular system of the plant and finally leading to the mortality of whole plant (Stover, 1962). The wilting symptom occur after 5-6 months of planting and symptoms get expressed both externally and internally (Wardlaw, 1961; Stover, 1962). Furthermore, the fungus survive in the agricultural soil as chlamydospores up to 30 years and thus poses a serious threat for banana production (Ploetz, 2000).

Although several strategies have been reported for combating the infection, cost effective management measures are still scantily available. Presently, available management strategies encompass crop rotation with rice, soil fumigation (Herbert and Marx, 1990) and fungicides (Lakshmanan et al., 1987). Nonetheless, efficient strategies in arresting the disease till recent past have been done by planting of resistant cultivars (Moore et al., 1999). Consumer preference limitations hinder the plantation of resistant varieties (Viljoen, 2002). Under these circumstances, the use of microbial antagonists that protect and promote plant growth by colonization and multiplication in the duality of locations could be an alternative strategy in the management of Fusarium wilt of banana.

Ample amount of research have been reported for the use of Arbuscular mycorrhizal (AM) fungi in reducing the incidence of plant root diseases caused by pathogens (Filion et al., 1999). The utilization of AMF in plant disease control has been well documented worldwide. The application of Glomus fasiculatum and Gigaspora margarita were found to decrease the root rot diseases caused by Fusarium oxysporum in Asparagus (Matsubara et al., 2001). Glomus clarum was reported to reduce the root necrosis caused by Rhizoctonia solani in cow pea (Abdel-Fattah and Shabana, 2002) and Glomus mossae systemically reduces the infection rate of Gaeumannomyces graminis in Barley (Khaosaad et al., 2007). Further, the AMF have been described to lessen the severity of disease caused by Foc and Cylindrocladium spathiphylli in banana plants (Jaizme-Vega et al., 1998; Declerck et al., 2002b).

Mycorrhizae Helper Bacterium (MHB) harboring the cytoplasm of many AMF spores (Cruz, 2004; Lumini et al., 2007) has exemplary resources in potentiating biocontrol of a wide variety of plant pathogens. Among which, Paenibacillus sp. is a
renowned MHB possessing extensive bioactive potentials against diverse fungal pathogens including *Phytophthora infestans, Fusarium oxysporum* and *Rhizoctonia solani* (Budi *et al.*, 1999). AM fungi-associated bacteria from the genus *Paenibacillus* sp. was found to habitat potent bioactives in hampering *Pythium* infections that is the causative agent of damping-off in cucumber (Li *et al.*, 2007).

For example, *Paenibacillus validus* supports the growth and sporulation of *Glomus intraradices* and this association is highly efficient in sustaining fungal growth and germination of new spores by release of sugars and some unidentified compounds in the rhizosphere of plant system (de Boer *et al.*, 2005). These findings indicated that AM fungus can grow independently in the host plant in the presence of their closely associated bacteria (Hildebrandt *et al.*, 2006). Till date, very few applications of AMF were reported for plant growth promotion and protection in banana such as *Glomus intraradices, G. mosseae* and *G. macrocarpum* (Jefwa *et al.*, 2010; Sukhada Mohandas *et al.*, 2010; Meenakumari *et al.*, 2017).

Therefore, in order to identify banana specific mycorrhizae, the AMF and its MHB in promoting the plant growth and also for tackling the problem of Fusarium wilt disease in banana, the following objectives were formulated.

**OBJECTIVES:**

1. To isolate and identify Arbuscular Mycorrhizal Fungi (AMF) and Mycorrhizae Helper Bacterial strains (MHB) isolates from different banana cultivars.
2. To characterise AMF and MHB isolates at molecular level.
3. To evaluate AMF along with MHB strains for the management of Fusarium Wilt disease in banana.