CHAPTER 1

INTRODUCTION
The term “pea” refers to small spherical seed or to the pod. Pea is a crop of high commercial significance. Pea belongs to the Leguminosae family, which has an important ecological advantage because it contributes to the development of low-input farming systems by fixing atmospheric nitrogen and it also minimizes the need for external inputs. Legumes constitute the third largest family of flowering plants, comprising more than 650 genera and 18,000 species. Around 200 years back Gregor John Mendel, known as father of modern genetics, conducted path breaking research in genetics using pea plant. Reasons for choosing pea include

1. Pea can be grown easily in large numbers.
2. The sexual reproduction is easy in pea.
3. The growth period is relatively short.

Its cultivation started in 3000 BC. The field pea is native to the Mediterranean region of Southern Europe. The important pea growing countries of the world are China, Russia, India, Ethiopia and U.S.A. In India, Uttar Pradesh is the major pea growing state. It is also grown in Punjab, Haryana, Delhi, Himachal Pradesh, Bihar, Maharashtra and Madhya Pradesh. The mature seeds are used as whole grain or split into 'dal'. Its dried grains are used as pulse and also as vegetable with potato in off-season after soaking in water for sometimes. Its green pods are one of the choicest vegetables. Green pods are dehydrated and canned and, thus, made available even in the off-season. Since pea is a leguminous crop, it is a rich source of protein.

**Pea (Pisum sativum)** is an important pulse crop. Pea requires cool growing season with moderate temperature. The best suitable temperature for its germination is about 22°C and 13°C to 18°C for plant growth and development. Pea requires less rain and low humidity for proper growth. Freshly opened flowers and pods are damaged by sudden change in temperature at flowering stage. The distinctive flower has 5 fused sepals, 5 petals, 10 stamens (9 fused in a staminal tube and 1 stamen is free), and one carpel, which develops into a pod with multiple peas. The average pea weighs 0.1 to 0.4 g. Pea crop can be grown in a variety of soils. A well drained soil with ample moisture retaining capacity such as deep loam soil is best for its cultivation. Light soils like sandy loam, are suitable for early crops especially for
green pod production. Heavy soils like clay loam are preferred for grain production. The pH range from 6 to 7.5 is the most suitable for this crop.

Peas are high in fibre, protein, vitamins (folate and vitamin C), minerals (iron, magnesium, phosphorus and zinc), and lutein (a yellow carotenoid pigment that benefits vision). Dry weight is about one-quarter protein and one-quarter carbohydrates (mostly sugars). Global production in 2009 of green peas was 16 million tons, harvested from 2.1 million hectares, with an additional 10.5 million tons of dried peas, from 6.2 million hectares. In some agricultural regions, such as the Punjab in India, peas are second only to wheat as a cultivated crop. Pea pods are botanically a fruit, but peas are called a vegetable in cooking. They are used as a vegetable, fresh, frozen or canned, and some varieties, such as split peas, are dried; these varieties are typically called field peas. Along with broad beans and lentils, pea was an important part of the diet of most people in the Middle East, North Africa and Europe during the middle ages. By the 17th and 18th centuries it had become popular to eat peas "green," or fresh, while they are immature and right after they are picked, especially in France and England.

Green peas are one of the main crops grown by farmers in Maharashtra during the rainy and winter seasons. The pea seeds are purchased mostly from dealers and not from other farmers or government agencies. This crop, being highly labour-intensive, will help to provide employment to the family members on the farm itself, particularly in the case of small and marginal farmers. It will provide impetus to the diversification programme of the state government, besides improving the soil health, being a leguminous crop.

**Mycorrhiza:**

Mycorrhizae are multifaceted associations comprising diverse morphological, functional and evolutionary categories (Smith and Read, 1997; Brundrett, 2002). Mycorrhizal interactions, especially arbuscular mycorrhizal (AM) interactions, occur in a wide variety of plants (Smith and Read, 1997). Root colonization by AM fungi is generally lower in nutrient-rich substrates than in nutrient-poor soils (Treseder, 2004). Arbuscular mycorrhizal colonization of plants may depend on edaphic properties and environmental factors, such as precipitation and sunlight hours. Arbuscular mycorrhizal colonization is negatively correlated with total N, total P, and available P
and soil organic matter but positively correlated with soil pH (Ling-Fei, et al., 2005; Kamareh et al., 2011). Mycorrhizal fungi are ubiquitous, present in all natural ecosystems in most climatic zones throughout the world. The mycorrhizal habitat is probably evolved as a survival mechanism for both partners in the association, allowing each to survive in environment of low fertility, drought, disease and temperature extremes where, alone they could not.

Early morphological classifications separated mycorrhiza into endomycorrhizal, ectomycorrhizal and ectendomycorrhizal associations based on the relative location of fungi in roots (Peyronel et al., 1969). These three types were not enough to describe the diversity of mycorrhizal associations. Harley and Smith (1983) had given the generally accepted classification. These include ectomycorrhizae, endomycorrhizae, and ectendomycorrhizae, arbutoid mycorrhizae, monotropoid mycorrhizae and orchid mycorrhizae.

**Ectomycorrhiza**

It is characterized by the presence of Hartig net, in which the hyphae of the fungi occupy between epidermis and the cortex cells without penetrating them. Hartig net consists of fan like or labyrinthine branch system providing a large surface of contact between the cells of the two symbionts. Many temperate shrubs and trees show short, dichotomous, swollen roots of yellow-green or orange-black colour. They are infected by ectomycorrhiza. *Pinus* is the best example of gymnosperm which does not survive without ectomycorrhiza. The symbionts belong to the group of higher fungi like Basidiomycotina and Ascomycotina.

**Endomycorrhiza**

It is characterized by the presence of finger like growth of arbuscules in the cortical cells of the host roots. For the same reason, this mycorrhiza is called as arbuscular mycorriza. Some of them produce swollen storage structures called as vesicles. e.g. *Acaulospora, Entrophospora* and *Glomus. Gigaspora* and *Scutellospora* produce only arbuscules. Endomycorrhiza is universal; but some families do not show mycorrhiza association. e.g. Chenopodiaceae and Cruciferae. This can be due to barriers in the cell wall or production of toxins by these plants or lack of essential nutrients. Endomycorrhizae have a unique ecological position in the rhizosphere.
region of the plant as they are partly inside and partly outside the host roots. The hyphae of the endophytes mostly occupy the intercellular spaces of the host tissue and after penetration of the cortical cells form a much divided haustorium, the arbuscule, intracellularly. The hyphae also develop vesicles inside the host cells and chlamydospores, azygospores, and sporocarps in soil.

**Ectendomycorrhiza**

This is basically an ectomycorrhiza having the arbuscules and vesicles like endomycorrhiza. Ectendomycorrhiza develop a well-organized Hartig net and the hyphae also penetrate inter- and intracellularly in the cortical regions of the roots. Hence, this is considered as the intermediate group. This type of mycorrhiza is observed in Arbutoideae and Ericaceae and also in the Pyrolaceae and Monotropaceae, all belonging to order Ericales (Harley and Smith, 1983).

**Arbutoid mycorrhiza**

This type of association is displayed by Arbutoideae family members and hence the name. This is characterized by the development of Hartig net and displays the coils of mycelium after penetrating the cortex cells of the short roots. The root system of Arbutoideae is heterorhizic and clearly differentiated into long and short roots. The long roots are sparsely infected in an intercellular fashion and contain a Hartig net e.g. *Pinus*

**Monotropoid mycorrhiza**

This type of association is found in members of family Monotropaceae and hence the name. The interaction between the host and the fungus is very peculiar in this case because unlike other plants, members of this family completely lack chlorophyll and hence they rely totally on the fungal partner for the carbon supply. The fungus produces the Hartig net and peg like structures attaching the cortex cells. The peg contents are emptied in the sacs of host membrane. The root system of *Monotropa hypopitys* forms a root ball. It shows extensive fungal mycelium ramified enclosing the roots.

**Ericoid mycorrhiza**
This type of association is found in the members of family Ericaceae and hence the name. Ericoid mycorrhizae never exhibit Hartig net but they do penetrate the cortical cells to produce the coils of vegetative mycelium. No arbuscular development is noted in this relationship. The hyphae only enter the cortical cells and do not enter the steler cells. Ericoid fungi that have been isolated in pure culture are not yet identified. But different studies agree that they must belong to or be closely related to the Ascomycetes species *Pezizella ericas*.

**Orchid mycorrhiza**

This type of association is found in the members of Orchidaceae family. In members of this family, mycorrhiza colonizes either the embryo in the seeds or achlorophyllous and chlorophyllous roots. Mycorrhiza penetrates the cells and form intracellular hyphal coils. Fungi that form mycorrhizal associations with orchidaceous plants are the genera belonging to the Basidiomycotina.

**Ophioglossoid mycorrhiza**

They occur in order Ophiglossales and considered as different because they develop freely in the soil even in the absence of host root. There is no morphological distinction into mycorrhizal and non mycorrhizal roots in members of Ophioglossaceae. The endophyte enters the root mechanically through the epiblemal layer without appresorium formation. The hyphae grow in the cortical region without penetrating meristematic zone, inner cortex and stele. The hyphae are septate and produce highly branched arbuscules, vesicles and hyphal swellings. At later stages, the hyphae clump in the host cells, lose their contents and fuse to form dark amorphous bodies. These bodies fuse to form large arbuscules (Nair, 2001).

Arbuscular mycorrhizal (AM) fungi are a type of endomycorrhiza. The diagnostic feature of arbuscular mycorrhizae (AM) is the development of a highly branched arbuscule within root cortical cells. The fungus initially grows between cortical cells, but soon penetrates the host cell wall and grows within the cell. As the fungus grows, the host cell membrane invaginates and envelopes the fungus, creating a new compartment where material of high molecular complexity is deposited. The AM fungi are the most intensively studied types of mycorrhizae because they are present in most agricultural and natural ecosystems and play an important role in plant
growth, health and productivity (Harley and Smith, 1983; Gianinazzi et al., 1990; Hosamani, 2005; Manimegalai et al., 2011; Kanchana and Gupta, 2012). There are only a few genera belonging to Brassicaceae; Chenopodiaceae and Cyperaceae where they are not found due to the presence of glucosinolates and their hydrolysis products isothiocyanates in and around the roots (Glenn et al., 1988; Hui, 2011), which are toxic to the growth of AM fungi.

**Morphotaxonomy**

The AM fungi have six genera belonging to the class Zygomycotina. All the six genera do not form vesicles (Morton, 1988). The different genera are distinguished by their spore colour, size, shape, hyphal attachment, hyphal length etc. The genera *Glomus* and *Sclerocystis* produce chlamydospores whereas *Acaulospora*, *Entrophospora*, *Gigaspora* and *Scutellospora* form azygospores. The AM fungi are placed under the order Glomales of class Zygomycotina by Morton and Benny (1990). The sub order Glomineae consists of the type family Glomaceae with two genera *Glomus* and *Sclerocystis*, characterized by singly borne chlamydospores or in compact sporocarps on one or more cylindrical to clavate subtending hyphae. The genera *Acaulospora* and *Entrophospora* are placed in Acaulosporaceae. They are characterized by the formation of 'chlamydospores' formed laterally from or within a hypha terminating in the soporiferous saccule. This family includes spore which show a progressive transformation from spores resembling *Glomus* to those which are uniquely sessile. The Gigasporaceae include two genera namely *Gigaspora* and *Scutellospora*. Fungi in the genera *Scutellospora* and *Gigaspora* have never been known to produce vesicles. In these, the azygospores produced are borne terminally on a sporogenous cell. Taxa included under sub order Gigasporineae produce auxiliary cells and no intraradical vesicles.

**Terminology of AM fungi**

**Appressoria:** Hyphal swellings between two adjacent root epidermal cells. These are sites where hyphae first penetrate root cells by exerting pressure and/or enzymatic activity.

**Arbuscules:** These are intricately branched "haustoria" that form within root cortex cells that look like little trees (Gallaud, 1905). Arbuscules are formed by repeated
dichotomous branching and reductions in hyphal width from an initial trunk hypha that ends in a proliferation of very fine branch hyphae. They are considered to be the major site of exchange with the host plant. Old arbuscules collapse progressively until only the trunk remains. Collapsed arbuscules are sometimes called peletons.

**Aseptate hyphae:** These are hyphae which are without cross walls (coenocytic hyphae). Cross walls may form as hyphae age.

**Auxiliary bodies:** These structures, which are also called external vesicles or accessory bodies, are clustered swellings on external hyphae. These are often ornamented by spines or knobs and are characteristic of *Scutellospora* and *Gigaspora*. These apparently do not function as propagules.

**Colony:** Hyphal colonization of a root resulting from one external hypha (these may arise from several adjacent entry points). These are often called infection units.

**Dichotomous branching:** A symmetrical branching pattern which occurs when two branches arise simultaneously from the tip of a hyphae, plant or fungus organ. Divergent branches grow at the same rate.

**Internal hyphae, intraradical hyphae:** Hyphae which grow within the cortex of a root to form a colony and later develop arbuscules and vesicles. These comprise the body (thallus) of a fungus in the root.

**Intercellular hyphae:** Hyphae which grow between the walls of adjacent root cells. These are in the root apoplast, the zone outside the cytoplasm of cells.

**Intracellular hyphae:** Hyphae which grow within root cells. These penetrate the walls of cells and grow within them, but are separated from the cytoplasm by the plasma membrane.

**Soil hyphae:** These are also known as extraradical or external hyphae and are the filamentous structures which comprise the fungal thallus (body) in the soil. They acquire nutrients, propagate the association, and produce spores and other structures. The AM fungi produce thick "runner" or "distributive" hyphae as well as thin, highly branched "absorptive" hyphae.
**Spores:** These are swollen structures with one or more subtending hyphae that form in the soil or in roots. Spores usually develop thick walls, which often have more than one layer. They can function as propagules. Spores of AM fungi are sometimes called chlamydospores or azygospores.

**Sporocarps:** Aggregations of spores into groups, which may contain specialised hyphae and can be encased in an outer layer (peridium). Soil particles may be included in the spore mass. This term can be misleading, as the sporocarps produced by most Glomeromycotan fungi are small and relatively unorganised structures compared to those produced by larger fungi.

**Vesicles:** Intercalary or terminal hyphal swellings formed on internal hyphae within the root cortex. These may form within or between cells. Vesicles accumulate lipids and may develop thick wall layers in older roots. The production and structure of vesicles varies between different genera of Glomeromycotan fungi. They are storage organs which may also function as propagules.

Aseptate hyphae spread along the cortex in both directions from the entry point to form a colony. Hyphae within root are initially without cross walls, but these may occur in older roots. Gallaud (1905) observed that VAM associations in different species formed two distinctive morphology types, which he named the **Arum** and **Paris** series after host plants. These are now known as **linear** and **coiling** associations respectively.

1. **Linear (Arum)** series associations where hyphae proliferate in the cortex by growing longitudinally between host cells. This occurs because hyphae grow through longitudinal intercellular air spaces that are present (Brundrett, 2004).

2. **Coiling (Paris)** series where hyphae spread by forming coils within cells because there are no continuous longitudinal air spaces.

**Ecology:**

Arbuscular mycorrhizal (AM) fungi are important components of virtually all terrestrial ecosystems and are especially critical in improving plant nutrient and water uptake under arid conditions (Allen, 1989; 2007; 2011). In some extreme environments, some plants cannot survive without AM fungi (Pennisi, 2004). AM
fungi also enhance plant competitive ability and determine community structure and ecosystem stability (van der Heijden et al., 1998; O'Connor et al., 2002; Hart et al., 2003). A number of studies have demonstrated that host plant species can affect the community composition and diversity of AM fungi (Johnson et al., 2004; Hausmann, 2009). Allen et al., (1984) first reported shifting phenology between C₃ and C₄ coexistence species. It has been reported that divergent phonologies may facilitate the coexistence of AM fungi in North Carolina grassland (Pringle and Bever, 2002). AM fungi can improve soil condition which contributes to plant growth. Soil factors also influence AM fungi diversity and development. Many studies have found that soil water and temperature can affect the mycorrhizae colonization, mycorrhizal production, and AM fungi community composition (Feil et al., 1988; Auge, 2001; Lekberg and Koide, 2008; Yamato, 2009).

AMF are of ecological and economical important as they can improve pathogen resistance (Vigo et al., 2000; de la Pena et al., 2006) as well as biomass production (Smith et al., 2009) of the host plant. In addition, AMF mitigate different kinds of plant stresses such as drought (Michelson and Rosendahl, 1990; Auge et al., 2001; Aroca et al., 2007), or heavy metal toxicity (Hildebrandt et al., 1999) and protect plants against root herbivores (Gange, 2001). Plant species richness often increases with the number of AMF species added in greenhouse experiments (Grime et al., 1987; van der Heijden et al., 1998, 2002, 2004) but can decline with AMF species richness in natural grasslands, based on comparisons after verses before fungicide treatment (Newsham et al., 1995; Hartnett and Wilson, 1999). These studies have led to models predicting that plant diversity should increase with AMF diversity (van der Heijden, 2002), or that AMF effects on plant diversity should depend on the mycorrhizal status of competitively dominant and subordinate plant species (Hartnett and Wilson, 2002; Allen et al., 2003; Urcelay and Diaz, 2003). AMF have been shown to improve productivity in soils of low fertility (Jeffries, 1987) and are particularly important for increasing the uptake of slowly diffusing ions such as PO₄³⁻ (Jacobsen et al., 1992), immobile nutrients such as P, Zn and Cu (Lambert et al., 1979; George et al., 1994; George et al., 1996; Ortas et al., 1996; Liu et al., 2002) and other nutrients such as Cadmium (Guo et al., 1996). Under drought conditions the uptake of highly mobile nutrients such as NO₃⁻ can also be enhanced by mycorrhizal associations (Ázcón et al., 1996; Subramanian and Charest, 1999).
AMF helps in promoting plant growth and development by increasing nutrient acquisition and alleviating stress conditions of plants (Koide and Kabir, 2000; Koide and Mosse, 2004; Barea et al., 2005). Also, AMF improve soil structure and play an important role in soil carbon (C) storage (Zhu and Miller, 2003; Rillig and Mummey, 2006). These roles of AMF have been linked with the production of a novel fungal substance, termed glomalin (Zhu and Miller, 2003; Rillig, 2004a, b). Glomalin, which is operationally defined and measured in soil as glomalin-related soil protein (GRSP), contributes to soil aggregate formation and stabilization due to its stability and hydrophobic nature (Wright and Upadhyaya, 1998, 1999; Rillig, 2004a, b). Also, there are accumulating reports that GRSP is a major pool of soil C and N (Rillig et al., 2001; Zhu and Miller, 2003; Nichols and Wright, 2006), thus glomalin can potentially reduce soil emissions of carbon dioxide (CO₂) and nitrous oxide (N₂O) into the atmosphere. This latter role of glomalin is of major interest as the reduction of atmospheric concentration of greenhouse gases is now of paramount importance (King, 2004). Wu et al., (2015) demonstrated that AM-mediated production of Glomalin-related soil protein (GRSP) and relevant soil enzyme activities may not depend on external P concentrations.

**IDENTIFICATION**

The morphology of resting spores is the main character which is used for identification of different genera and species. Most of the spores are asexually produced chlamydospores, whereas some are azygospores. These azygospores are distinguished by the presence of a bulbous attachment, referred to as the vestigial gametangium.

**Size, shape and colour of the spore:** The spores of AM fungi have an average diameter of 100-250 μm; however the range occurs in between 50 μm and 500 μm. The spores can be spherical or oval and regular or irregular in shape. The colour of spores of AM fungi may be reddish brown (specially old) yellowish brown (common) grayish brown, honey coloured, amber pale, greenish yellow or colorless (hyaline). The spore contents should change with age.

**Cytoplasmic structure:** Cytoplasm is either reticulate having very fine beaded strands or vacuolated consisting homogeneous system having lipid droplets of different size (Mosse and Bowen, 1968). Reticulate arrangements indicates membrane
bounded fat deposit do coalesce. Sometimes due to pressure within the spore content may change to polygonal shape.

**Hyphal attachment:** Hyphal attachment is an important characteristic of morphological classification. Most of the spores are born either at terminal or intercalary position. Hyphal attachment AM fungi differentiates six genera from each other (Morton and Benny, 1990). *Glomus* and *Sclerocystis* with simple attachments, while *Gigaspora* and *Scutellospora* with bulbous attachments and *Acaulospora* and *Entrophospora* without attachments.

**Structure of the wall:** Generally the wall is thick chitinous and brittle. In some spores the wall is simple, whereas in others it is multilayered. Two layered wall is more common in occurrence in some the inner wall is folded. There are spores having several layered laminated wall where the innermost layer is the thickest and more densely colored.

**Germination:** The germination of spore may be by one germ tube through the main stalk; in spores with vacuolated cytoplasm where as spores with reticulate cytoplasm have several germ tubes. The plant root exudates are essential for mycorrhizal germination. They are produced by several pathways and biochemical mechanisms. They contain polysaccharides, sugars, organic acids, amino acids, and phenolics, enzymes like acid phosphatase and volatile substances like ethylene. They alter the physical and chemical conditions of rhizosphere and change the pH and mineral availability. The phosphate status of the plant acts as a signal for mycorrhizal formation (Graham *et al.*, 1981).

**Applications of AM fungi**

1. Arbuscular mycorrhizal fungi (AMF) are important for plant growth and nutrition. There is potential to use AMF inoculum to enhance the use efficiency of P fertilizers. Inoculation with AMF affects plant P content with or without P fertilizer addition. Inoculation may improve P availability in P unamended and amended soil.

2. Arbuscular mycorrhizal (AM) fungi have an extraordinary importance since they increase nutrient acquisition by the plant as well as resistance to biotic
and abiotic stress. The symbiosis with AM fungi has been proposed as one of the mechanisms of heavy metal plant tolerance and water stress avoidance.

3. The mycorrhizal symbiosis maintains and, in many cases, stimulates plant growth while substantially reducing fertilizer requirements. The fungal mycelium acts for plants as an extension of their root systems, allowing them to optimize the use of soil water and soil minerals from a much larger volume of soil. Healthier plants better resist environmental stresses such as drought, chilling and pollution, and have an improved capacity to survive certain bacterial and fungal pathogen attacks.

4. The AM fungi improve soil quality by increasing soil micro flora diversity and abundance and by reducing soil erosion through a better plant rooting capacity.

5. The arbuscular mycorrhizal association in plant root improves resistance to root rot and collar rot diseases.

6. Mycorrhiza can be used for ex vitro establishment of tissue culture plantlets.

7. Mycorrhiza plays an important role in nutrient cycling and stabilization and restoration of ecosystem.

8. Mycorrhizal symbiosis can be used as a model for understanding other types of plant microbe associations.

9. Mycorrhiza may enhance the ability of plant to cope with water stress situations associated to nutrient deficiency and drought.

**AM fungi and Rhizobium in legumes**

Naturally occurring plant-AMF combinations may indicate functional relationships, and different plant types may host different AMF communities. Legumes form a special plant functional group due to their symbiosis with rhizobia, which can fix atmospheric nitrogen (Sprent, 2001). Legumes contribute substantially to nitrogen input and the productivity of many terrestrial ecosystems (Cleveland *et al.*, 1999 and Vendemeer, 1989). Rhizobial nitrogen fixation provides legumes with an additional nitrogen source, but it requires large amounts of energy and phosphorus
(Almeida et al., 2000 and Sa et al., 1991). The amount of phosphorus delivered by different AMF species varies and legumes might preferentially associate with specific AMF that are efficient in supplying phosphorus. AMF also enhance nodulation and nitrogen fixation, but the extent of these effects is dependent on the AMF species.

The AM fungi are cornerstone of the development of sustainable agriculture. As such, there is a necessity to accelerate their integration in agriculture production systems. The constant increase in food demand throughout the world, the rise of biofuels production and the imminent depletion of phosphate stock provisioning strengthen the necessity to seriously support research on mycorrhizal symbioses both in India as well as internationally. There is a limited research work done on the association of pea and mycorrhiza and there are fewer reports showing the effect of water stress, salt stress and AM fungi on pea and careful co-selection of VAM species and *Rhizobium* strains can enhance pea yield and nutrition. Hence present work was undertaken by selecting pea plant which is one of the important cash crops from Maharashtra, India.

**The Aims and Objectives of present Investigation are as follows:**

1. To isolate and identify AM fungi from rhizosphere of pea from selected localities of Purander taluka of Pune district.
2. To study the effect of various AM fungi on drought and salt tolerance, growth and productivity in pea.
3. To study the interaction of AM fungi with *Rhizobium* of pea.
4. To check the effect of AM fungi on biochemical contents of pea.
5. To produce inoculum on large scale by using suitable host plants like jowar.

To study the plant root and fungal association, relevant literature has been surveyed thoroughly which is used to design the approach for study of AM fungi and its developments.