CHAPTER 2

Literature Review

2.1 Botanical characteristics of *C. alismatifolia* Gagnep.

C. alismatifolia Gagnep. belongs to the family Zingiberaceae, subgenus Paracurcuma. It is native to the Indo Malayan region, as herbaceous ornamentals, an economically important bulbous crop for rhizome production, cut flowers, pot plants (Bailey, 1925). The growth cycle starts from April to May, plants flower from June to August during the rainy season. The average temperature is 29˚ - 30˚C with 80% relative humidity, 13 hr day length and under 30% - 50% natural radiant flux density. And then, rhizome becomes dormant from November to December during winter season (Lawson and Roh, 1992; Roh *et al*., 2006; Wichailak, 2006).

Root system

The root system of *C. alismatifolia* has two types, i.e., fibrous roots and contractile roots. Fibrous roots initiate and develop from storage roots and the base of new shoot as soon as a rhizome sprouts. The contractile roots are thickened specialized roots, initiate from the base of new shoot and develop into storage roots

(Figure 2.1, B). It is thought to act as storage organs and played an important role in growth and development (Hagiladi *et al*., 1997).

Rhizome

The rhizome is modified from stem that typically has short internodes (stubbed rhizome), ovule shaped and it is formed from lateral buds at the base of the pseudostem when the plant is mature (Phongpreecha, 1997). The induction stage of new rhizome formation may be started at 11-13 weeks after planting (WAP) when vegetative growth is maximal. Differentiation and development stage continuously occurre during 14-22 WAP and rhizome is ripened at 23 WAP when its growth is terminated (Chidburee *et al*., 2009).

Leaves

The leaves of *C. alismatifolia* consist of leaf sheath and leaf blade (Figure 2.1 C). The leaf sheath is tightly wrapped and compact, so called pseudostem. The leaves are alternate and distichous, the base sheathing and the blade are mostly elliptic with penni-parallel, strongly ascending veins (Gerald, 1997).

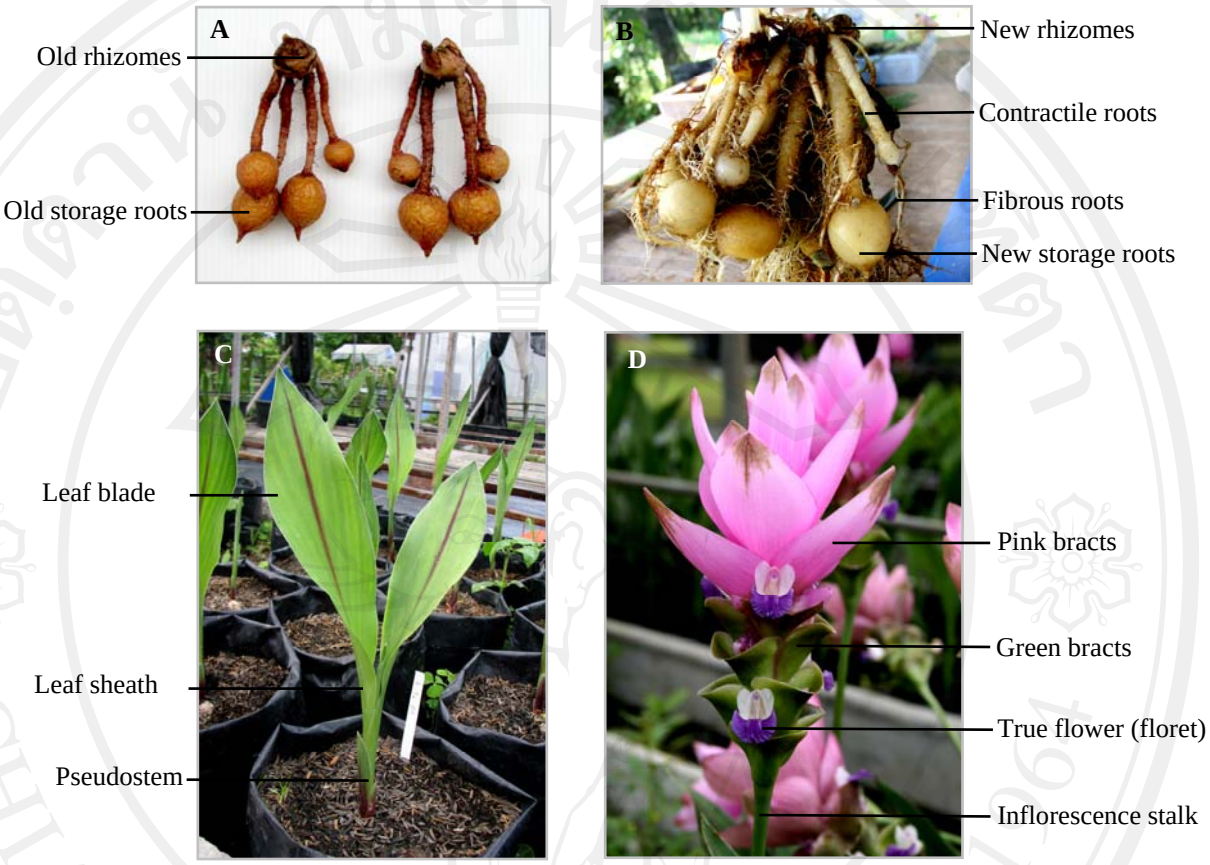

Figure 2.1 Morphology of *Curcuma alismatifolia* Gagnep.;

A : old rhizomes and old storage roots, B : new rhizomes and new storage roots,

C : leaf blade and D : inflorescence

Inflorescence

The inflorescence is lotus-shaped (Figure 2.1, D), showy compact spike and a long post harvest vase life (Roh *et al*., 2006). Its vase life is approximately 5 days, determined by browning and wilting of the coma bracts. This browning may be related to ethylene production (Bunya-Atichart *et al*., 2004). Inflorescence comprises a number of pink coma bracts in upper part and green coma bracts in the lower part,

with small true flower (floret) (Hagiladi *et al*., 1997). True flower has no pedicel. It is composed of 3 calyx tube sepals and 3 petals (Lekawatana and Pituck, 1998).

Flower

True flowers that hide in the axils of the green bracts are bisexual, strongly zygomorphic, and often are associated with conspicuous floral bracts in a spike. The perianth is in 2 whorls, and herbaceous or membranous 3-lobed or spathaceous tubular calyx and a petaloid tubular corolla with 3 lobes. The androecium typically consists of 1 fertile stamen, a large opposing petaloid labellum representing 2 connate staminodia, and 2 smaller flanking petaloid staminodia. The gynoecium consists of a single compound pistil of 3 carpels, a single style nestled in a channel of the filament and anther of the fertile stamen and inferior ovary with typically 3 locules, each containing numerous axile ovules. Rarely the ovary is unilocular with parietal placentation (Gerald, 1997).

2.2 *C. alismatifolia* production

Curcuma production is of two methods, i.e., 1) planting in the field and 2) grown in plastic bags. The production area for field production should not be rotated with following crops, i.e., tomato, sharrow, ginger, etc. at least for three years. Plastic bag planting usually use soilless media such as sand:rice husk:rice husk charcoal at a ratio 1:1:1. Growing media should be steriled by solar irradiation at least 1 month before using. Plastic bag size is 15x30 cm and placed on a clear plastic sheet (Department of Agriculture, 2006).

Generally, planting starts in rainy season (April to May) using rhizome, with 4-5 storage roots. The vegetative shoot sprouts from lateral bud, at 1-2 week after planting (WAP). Shoot grows and develops to 1st foliage leaf at 2-3 WAP. Generally, plant produces 3-5 fully expanded leaves per shoot before flowering at 8-10 WAP. When they are 11-15 cm in height, the plants start to differentiate the inflorescence by enlarging bud in bract axil and depressing flower of primordium center for first true flower (floret) initiation. The 1st floret is opened at 10 WAP and fully bloomed at 11-12 WAP. The base of flower stalk is connected with a new rhizome which is developed from the basal leaf sheath. The first inflorescence ceases when 1-3 new shoots per plant are produced and the terminal of contractile roots become swollen, and develop into new storage roots which is caused by cell division and cell enlargement in the cortex and stele. New rhizomes, with attached storage roots, are harvested. One old rhizome can produce up to 4-5 new rhizomes with new storage roots (Changjeraja *et al*., 2009; Chidburee *et al*., 2009; Hongpakdee *et al*., 2010).

2.3 Tissues culture production

In vitro regeneration of *Curcuma* is achieved through shoot meristem culture. The shoot buds (2-3 cm long) from rhizome are inoculated on MS medium supplemented with 3.0 mg/l BAP for initiation and elongation of shoot (Shukla *et al*., 2007). Recently, Topoonyanont *et al*. (2005) reported success with micro propagation of *C. alismatifolia* from dwarf shoots, which were termed retarded shoots. Morphologically, leaf and stem expansion of the retarded shoots are reduced, but the stem and the shoot are maintained and can be seen with the naked eye. A popular

starting part for culturing *C. alismatifolia* Gagnep. by tissue culture method is the immature flower inflorescence that begins to emerge from pseudostem where it is still covered with bracts. Approximately, 1.0 cm long of the inflorescence is excised and cultured in MS cultured media adding BA and young coconut juice. The sprout from this culturing technique takes 2 years to flower and produces flowering size of rhizome (Wannakrairoj, 1996).

2.4 Effects of fertilizer on growth of *Curcuma*

For *C. alismatifolia*, supply of optimum fertilizer application rate (7.5 g/plant) was recommended. The high nitrogen content such as 15-0-0 or 21-7-14 from the two-leaf fully-expanded stage until the flowering stage and then of 13-13-21 fertilizers to produce good rhizome quality (Siritrakulsak, 2010). Ohtake *et al.* (2006) reported that increased nitrogen application at 50 mg/l could increase the number of flowering shoots and, consequently, the number of rhizomes. Based on the production of rhizomes with four to six tuberous roots, optimum concentration of 15-7-14 water-soluble fertilizer was 2.7 g/l for *C. alismatifolia* 'Chiang Mai Pink' and 1.3 to 4.0 g/l for *C. thorelii* 'Chiang Mai Snow'. Ruamrungsri and Apavatjut (2003) reported that N played an important role for growth and quality of *Curcuma* rhizomes and inflorescence. A stubbed rhizome of *C. alismatifolia* accumulated about 4-5% of N based on dry weight and 97% of N was insoluble form. Storage roots stored only about 1-2% N at the beginning of dormancy and 88% was most abundant in insoluble from (Ruamrungsri *et al*., 2010).

2.5 Biofertilizer use in agricultural crops

Biofertilizer is a ready-to-use live formulation of such beneficial microorganisms which on application to seed, root or soil, mobilize the availability of nutrients by their biological activity. There are 3 types of biofertilizers, i.e., 1) nitrogen-fixing biofertilizers which include 2 subtypes, 1) symbiotic N-fixing e.g. *Rhizobium*, *Actinomycetes*, *Anabaena* and 2) non-symbiotic N-fixing, e.g., *Azotobacter*, *Azospirillum*, blue green algae, 2) phosphate biofertilizers, including 2 subtypes, 1) P-solubilizing, e.g., *Bacillus*, *Pseudomonus*, fungi and 2) P-absorbing e.g. AMF. The last is cellulolytic biofertilizers. The biofertilizers have many benefits in crops, they can increase crop yield by 15-20% and decrease consumption of fertilizer. Besides, they improves soil properties and sustain soil fertility, help in mineralization of plant nutrients, solublize unavailable phosphate and sulfur in the soil converting them into available form. Traditionally, farmers and gardeners rely on chemical fertilizers to restore nutrients to the soil. However, over utilization of chemical fertilizers pollute surface and ground waters, destroy helpful microorganisms in the soil and increase susceptibility to plant diseases. Biofertilizers, in contrast, use natural biological wastes to boost soil fertility without the harmful effects (Verma and Bhattacharya, 1990; Kaur *et al*., 2011).

2.6 Endophytic diazotrophic bacteria (EDB)

Endophyte simply means the location of an organism, with “endo” means “inside” and “phyte” means “plants”. Therefore, endophyte refers to organisms that live within plants (Wilson, 1995). The EDB living within plant tissue as these bacteria can be either obligate or facultative. Some EDB are able to colonize thousands of different plant species, while some are restricted to plant families. Some groups of EDB have been believed to be mutualists that protect plants against biotic stresses. Co-evolution may exist between endophytes and their host in resistance to environmental stresses. During the last two decades, endophytes have been targeted as valuable sources of new bioactive compounds (Tadych and White, 2009). EDB are detected from inside surface-disinfested plants or extracted from inside plants and have no visibly harmful effects on the plants (Hallmann *et al*., 1997). Inside the plant tissue, the density of EDB is less than rhizospheric bacteria and bacterial pathogens (Guo *et al*., 2008).

Some of the endophytes are proved to be able to enhance plant growth by nitrogen fixation, increase resistance against pathogens, remove contaminants, solubilize phosphate, produce a wide range of phytohormones; such as auxins, cytokinins and the gibberellins, and produce antibiotic agents (Hornschuh *et al*., 2002). Some bacterial endophytes are originally from the phyllosphere bacterial communities in phyllophane, endophyte infected seeds and plant materials. Endophytic microorganisms depend on the nutrient supplied by host plants, so parameters which affect plant nutrient supplies will consequently influence endophytic communities. Thus, physical factors, such as temperature, rainfall and UV

radiation, will affect endophytic communities indirectly. In addition, soil physical and chemical factors also have an indirect effect on the endophytic communities. The factors, including pH, salinity and soil texture can alter the saprophytic bacteria in rhizosphere, resulting in preselecting the endophytic bacterial source (Faeth and Fagan, 2002).

2.6.1 Nitrogen fixation by EDB

Nitrogen fixation by EDB occurs by converting N_2 to NH_3, using nitrogenase enzyme. The ability to fix atmospheric N_2 of diazotroph is involved with the nitrogenase and genetic expression of the nitrogen-fixation (nif) gene. The nitrogenase enzyme consists of two components. Both components form aggregates and can be shown to consist of subunits: The component I, a dinitrogenase or molybdoprotein (MoFe protein). The component II, a dinitrogenase reductase or iron protein (Fe protein) (Figure 2.2). These protein components are irreversibly inactivated by oxygen. A cofactor containing Mo, Fe and S, helps in the activity of nitrogenase and can be separated from the MoFe protein. Both a reductant and ATP are required for enzyme activity; approximately 16 moles of ATP are consumed per mole of dinitrogen reduced (Rubio and Ludden, 2005).

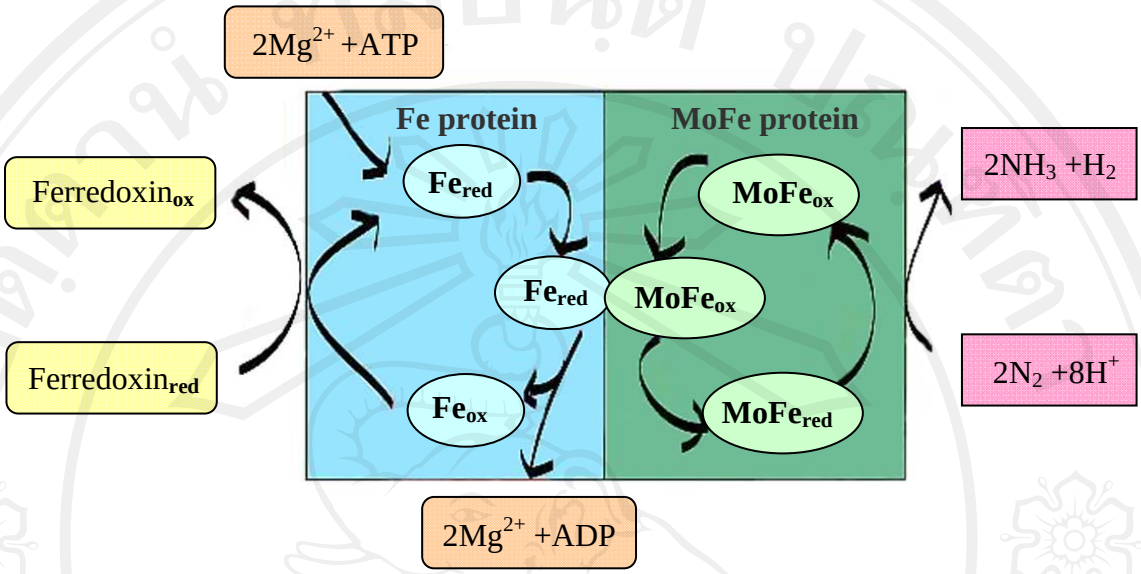

Figure 2.2 The two component structures of nitrogenase enzyme

Mechanism of nitrogenase

Nitrogenase reduces many small molecules with triple bonds in addition to nitrogen. Oxygen, which is triple-bonded inactivates nitrogenase. Carbon monoxide, another triple-bonded molecule, is a competitive inhibitor (Deacon, 2005).

Nitrogen is reduced at the MoFe cofactor site on the molybdoferredoxin. The intermediates N_2H_2 and N_2H_4 (hydrazine) are assumed to exist. Although N_2H_4 has been detected, N_2H_2 is very unstable and tended to decompose back to N_2+H_2 (Deacon, 2005).

Hydrogen is always produced when nitrogenase reduces N_2 to NH_3. There are two views on this. The first view is that, this is a side reaction-nitrogenase, a powerful reductant that the conversion of H_2O to H_2 inevitably occurs. The second view is that

under optimum conditions, one H_2 is evolved per N_2 fixed, suggesting that H_2 evolution is an internal part of the enzyme mechanism (Deacon, 2005).

Biological nitrogen fixation can be determined by several methods. The common method for measuring nitrogen fixation is acetylene reduction assays (ARA). It is an indirect method, more convenient and time saving than direct method, such as use of the radioactive isotope of nitrogen (^{15}N). During nitrogen fixation, there are two electrons transferred to ethylene as shown in the equation below (Vangnai, 1998; Giller and Wilson, 1991).

$$\underset{\text{(acetylene)}}{C_2H_2} + 2H^+ + 2e^- \longrightarrow \underset{\text{(ethylene)}}{C_2H_4}$$

Therefore, the acetylene was reduced to ethylene every time nitrogen was fixed. The amount of ethylene could be measured by gas chromatography (Vangnai, 1998)

2.6.2 Identification of EDB by 16S rDNA sequencing

The 16S ribosomal DNA gene is a section of prokaryotic DNA found in all bacteria. The 16S rDNA sequence is a gene encoding small subunit ribosomal RNA. This gene codes for an rRNA, and this rRNA in turn makes up part of the ribosome. The ribosome is composed of two subunits, the large subunit (LSU) and the small subunit (SSU). These two subunits sandwich the mRNA as it feeds through the ribosome for translation. Ribosomes have mostly conserved the important function over time, that their structure has changed very little. There are parts that have been

conserved more than others (conserved regions) while other portions are looped and unbounded (hypervariable regions). Primers are designed to bind to conserved regions and amplify variable regions. The DNA sequence of 16S rDNA gene has been determined for an extremely large number of species (Lane, 1991; Green gene, 2011).

New and exciting molecular methods, many using the 16S small subunit ribosomal nucleic acid molecule, are opening the microbial black box in soil. These studies have added much to our knowledge of microbial diversity in soils, and are beginning to advance our understanding of the relationship between this diversity and its function in soil processes (Macrae, 2000). Another benefit of the widespread use of 16S rDNA technique to survey bacterial diversity in different soils is that a number of taxa, common in geographically-distinct soil, have been identified (Ludwig *et al*., 1997).

2.6.3 Effects of diazotrophic bacteria on plant growth

Diazotrophic bacteria are ubiquitous in most plant species, residing latently or actively colonizing plant tissues. Some diazotrophic bacteria have been found to possess several beneficial effects on host plants, such as plant growth stimulation, nitrogen fixation, producing indole-3-acetic acid (IAA), mycorrhizal colonization (Will and Sylvia, 1990), induction of resistance against plant pathogens (Elbeltagy *et al*., 2002), P- solubilization (Freitas *et al*., 1996), production of antibiotics (Rosado and Seldin, 1993), cytokinin (Timmusk *et al*., 1999), hydrolytic enzymes (Nielsen and Sørensen, 1997) and increase root and shoot growth of crop (Sudha *et al*., 1999). *Pseudomonas* inoculants have been reported to significantly increase root dry weight in spring wheat (Walley and Germida, 1997), yield in sugar beet (Çakmakçi *et al*.,

2001), colonized winter wheat roots (Freitas and Germida, 1992), could effectively adapt to new environments (Misko and Germida, 2002) and promote the growth of the spinach (Urashima and Hori, 2003). Some species, such as *Pseudomonas fluorescens*, *Curtobacterium luteum* and *Bacillus amyloliquefaciens*, have been reported to control plant pathogenic bacteria like *Clavibacter michiganensis* and *Erwinnia carotovora* (Buren *et al*., 1993; Sturz and Matheson, 1996). Many reports have suggested that plant growth could be promoted by some endophytic bacteria related to N_2 fixation and phytohormone synthesis (Bandara *et al*., 2006). The N_2-fixing rate differs among plant species, such as rice (12-74 µmol C_2H_4/plant/24hr), wheat (0.6-3.1 µmol C_2H_4/plant/24hr), and *Dendrobium crystallinum* (0.02-6.23 µmol C_2H_4/plant/24hr) (Anwar, 1999; Chuanchaisit, 2006). Regarding auxin production by endophytic bacteria, there have been many reports of its presence involving various plant species, such as *Calanthe vestita* (1.18-6.60 µg IAA/ml) and *Azolla filiculoides* (1.5-10.1 µg IAA/ml) (Tsavkelova *et al*., 2005; Forni *et al*., 1992).

The nitrogen-fixation bacteria associated with candidate bio-fuel plants need to be identified, and it is also essential to evaluate their performance in plant. *Miscanthus* and switch grass are two of the most promising bio-fuel plants that have been planted widely in Europe and the United States (Lewandowski *et al*., 2003)

2.7 Arbuscular mycorrhizal fungi (AMF)

2.7.1 Characteristics of AMF

AMF has been originally referred to as vesicular-arbuscular mycorrhizas, a name is still used, are mutualistic symbiotic associations between the roots of most vascular plants and a small group of fungi in the phylum Glomeromycota (Schüßler *et al*., 2001a). Although some structural variation exists in this category, most arbuscular mycorrhizas are characterized by the presence of intraradical hyphae (intercellular or intracellular in location), arbuscules (finely-branched hyphae involved in nutrient exchange), extraradical mycelim (hyphae that connect the roots to the soil), and spores formed in the extraradical mycelium. (Figure 2.3). Some fungal species also form intraradical structures referred to as vesicles, such as species in the genera *Gigaspora* and *Scutellospora* (Peterson *et al*., 2004).

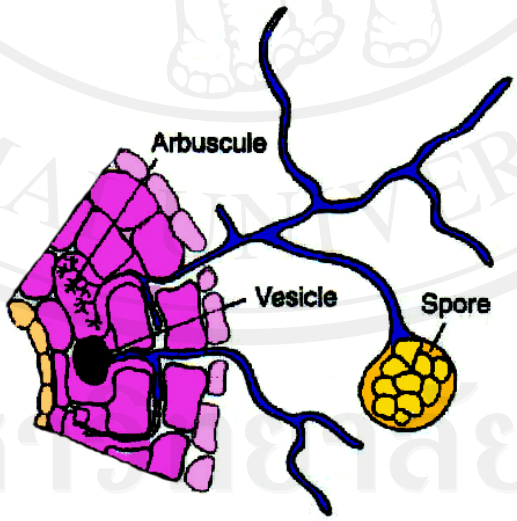

Figure 2.3 Characteristics of AMF

Arbuscular mycorrhizal fungi structures

Hyphae

Non-septate hyphae which grow within the wall of root cortex cells and a network of hyphae forms in the soil with thicker hyphae function as conducts and thin branched hyphae are thought to absorb nutrients. Gallaud (1905) observed differences of hyphal form in cortex of different host plant species which have been divided into 2 types: *Arum* and *Paris*-type. The *Arum*-type is usually a single branch from either an intercellular or intracellular hyphae narrows and penetrates the wall of a cortical cell and forms a trunk hyphae which branches repeatedly to form a complex tree-like structure, the arbuscule. The *Paris*-type only presents in intracellular hyphae. These develop complex coils from which fine lateral branches are initiated (Figure 2.4) (Peterson *et al*., 2004).

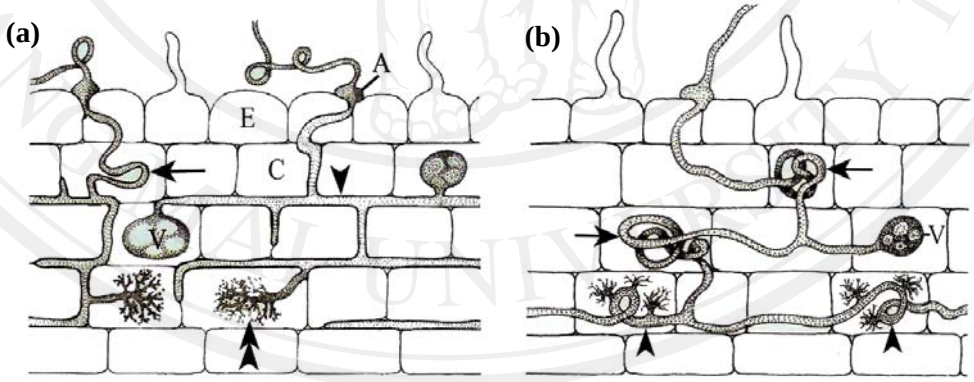

Figure 2.4 Characterization of hyphae AMF (Peterson *et al*., 2004).

(a) : *Arum*-type AMF association ; (b) :*Paris*-type AMF;

A = appressoria ; E = epidermal cell ; C = cortex ; arrow = a hypha forms a coil ;

arrowhead = intercellular space hyphae ; double arrowheads = arbuscules ;

V = vesicles, association ; arrows = hyphae coiling ; arrowhead = arbusculate coils

Arbuscules

Arbuscules are intricately branched within root cortex cells. Thses are the major site of symbiotic exchange with host plants. Furthermore, there are intense alkaline phosphatase and possible ATPase activities (Peterson *et al*., 2004).

Vesicles

Vesicles look like the intramatrical spores. These are globose bodies caused by terminal hyphal swellings formed on inter- or intracellular hyphae. Vesicles are storage structures with accumulated mostly lipids (Peterson *et al*., 2004).

Auxiliary bodies

Also called external vesicles, auxiliary bodies are clustered swellings on external hyphae. These structures are characteristics of *Gigaspora* and *Scutellospora.* Auxiliary bodies cannot function as propagules (Peterson *et al*., 2004).

Spores

Asexual structures (20->1,000 µm diameter) that form as swelling on one or more subtending hyphae in soil, sometimes found in roots. Spores can function as propagules and size, color and wall layers are used to classify species (Peterson *et al*., 2004).

2.7.2 Taxonomy of AMF

AMF are the least diverse group of mycorrhizal fungi on a species level. There are more than 160 species of AMF (Morton and Benny, 1990). The classification of Glomeromycota by Morton and Redecker (2001) was placed in the following; one order (Glomerales); five families (Acaulosporaceae, Archaeosporaceae, Gigasporaceae, Glomeraceae, and Paraglomeraceae), seven genera (*Acaulospora, Archaeospora, Entrophospora, Gigaspora, Glomus, Paraglomus* and *Scutellospora*) (Figure 2.5). Until now, there are ten genera, eight families and four orders in phylum Glomeromycota (Walker and Schüßler, 2004). The revision of the taxonomy of AMF has been continued.

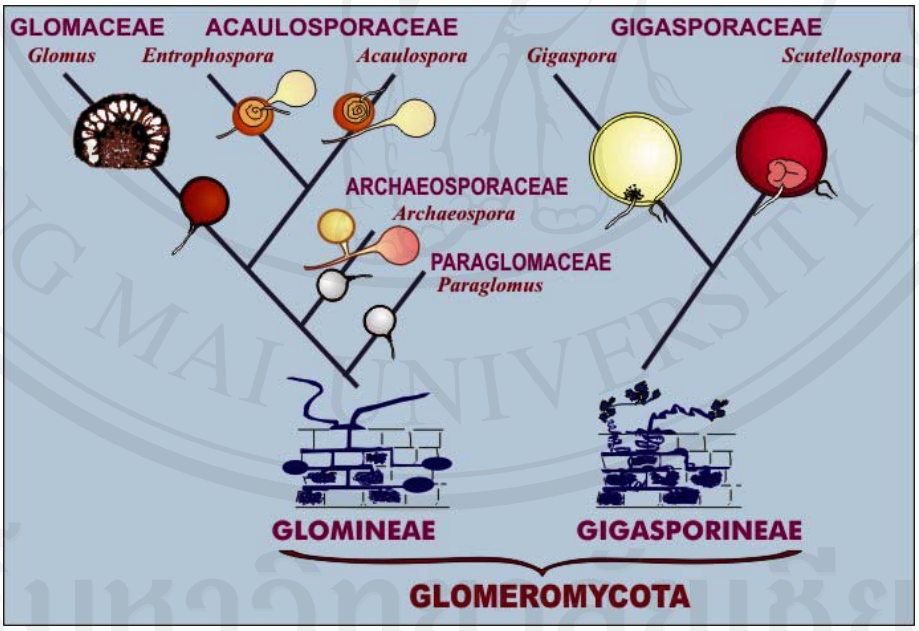

Figure 2.5 Classification of Glomeromycota (Invam, 2011)

The process of spore formation has been important to circumscribe genera and families, and the spore features to distinguish species. Moreover, biochemical properties have also provided valuable information for the classification based on serology, isozyme variation and fatty acid variation methods (Brudrett *et al*., 1996; Koide and Mosses, 2004). Since molecular phylogenetic methods have been used to elucidate the phylogenetic relationships among these fungi, it is amazing the extent to which morphological and molecular methods have yielded similar results. Thus, identification of AMF will probably continue to be based primarily on structural characters and considered a consensus of morphological and molecular evidence will be important. However, even the identification of healthy spores may pose problems, because morphological characters are scarce in some species of AMF. Some AMF species cannot be discerned by spore morphology (Koide and Mosses, 2004). Thus, molecular identification techniques have the potential to classify AMF. Many researches of molecular identification of AMF have targeted parts of ribosomal gene (rDNA), such as 18S small subunit sequences (SSU) (Simon *et al*., 1992; Schüßler *et al*., 2001b), 28S large subunit sequence (LSU) (Silva *et al*., 2006), and both small and large subunits (SSU and LSU) (Renker *et al*., 2003). The rDNA gene contains highly conserved coding regions (18S, 5.8S and 28S). These regions are separated by 2 sequences, which are intragenic transcribed spacer (ITS) and intergenic spacer (IGS) (Figure 2.6). These ITS regions indicates that there are more variations and differences in species level (Gardes and Bruns, 1993). Molecular characterization of AMF is in most case achieved by polymerase chain reaction (PCR) on DNA from roots of host plants, spores or soil samples (Krüger *et al*., 2009). Furthermore, having described a method that allows quick and easy PCR amplification and cloning of

nearly complete SSU rDNA gene from AMF, the SSU rDNA has been extensively used in fungal taxanomy and biodiversity studies (Bruns *et al*., 1992).

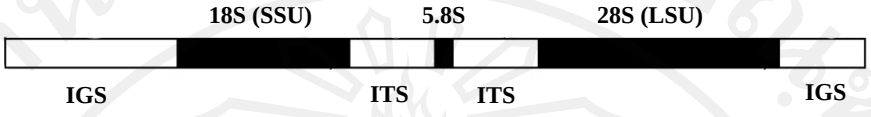

Figure 2.6 Structure of the ribosomal gene (rDNA)

unit contains the 18S, 5.8S and 28S genes (Gardes and Bruns, 1993).

2.7.3 Role of AMF to plant

Hyphae of mycorrhizal fungi have the potential to greatly increase the absorbing surface area of the roots, the hyphae must be distributed beyond the nutrient depletion zone that develops around the roots. A nutrient depletion zone develops when nutrients are removed from the soil solution more rapidly than they can be replaced by diffusion (Rousseau *et al*., 1994). Furthermore, narrow hyphae can grow into small soil pores inaccessible to roots or even root hairs. Another advantage attributed to mycorrhizal fungi is access to pools of phosphorus not readily available to the plant. One mechanism for this access is the physicochemical release of inorganic and organic phosphorus by organic acids through the action of low-molecular-weight organic anion such as oxalate which can: (1) replace phosphorus sorbet at metal-hydroxide surfaces through ligand-exchange reactions, (2) dissolve metal-oxide surfaces that absorb phosphorus, and (3) complex metals in a solution and thus prevent precipitation of the metal phosphates (Fox *et al*., 1990).

The benefits of AMF to plants are well-known, including enhanced uptake of water and soil nutrients, such as N, K, Ca, Mg, Cu, Mn, Zn and, especially, P (Marschner and Dell, 1994). For example, Shibata and Yano (2003) examined P acquisition from non-labile phosphate (Fe-P, Al-P and Ca-phytate) in peanut (*Arachis hypogea* L.) and pigeonpea (*Cajanus cajan* L.) with mycorrhizal interaction. Inoculation with *Gigaspora margarita* greatly increased P uptake in peanut (6-fold) and pigeonpea (10-fold). The result revealed that mycorrhiza could accelerate P acquisition by both of these plants from non-labile sources in P-limited soil. Rutto *et al*. (2002) evaluated the effect of *Gigaspora margarita* on mineral status of peach seedling. Mycorrhizal seedlings showed significantly higher concentrations of shoot P, K, and Zn. The combination treatment of AMF and rock phosphate have the potential to increase plant growth where phosphorus was limiting plant production (Dodd *et al*., 1990). Nye (1997) found that *Glomus intraradices* could uptake nitrogen, phosphorus and potassium from soil and transport it to the host plant and increase significantly the shoot biomass yield. Rajan *et al*. (2000) reported that *Glomus leptotrichum* showed the best efficiency among nine AMF in increasing growth and nutrients (P, Zn and Cu). AMF could enhance seeding vigor of cashew nut inoculated with *Glomus faciculatum* which increased shoot length, internode length, internode number, number of leaves, stem diameter, root number and root length than uninoculated plants (Ananthakrishnan *et al*., 2004). Mycorrhiza also were known to reduce problems with pathogens which attack the roots of plants (Gianinazzi-Pearson and Gianinazzi, 1983). Colonization of tomato root by *Glomus mosseae* compensated for the reduction of plant growth by *Meloidogyne incogyne* infection (Talavera *et al*., 2001).

2.7.4 Interaction of AMF with other soil microorganisms

AMF also interact with a whole range of other microorganisms in soil (Johansson *et al*., 2004). The activity modification of plant-microbe interactions such as the mycorrhiza or the *Rhizobium* symbiosis or by inducing changes in the microbial population balance that had beneficial rhizosphere microorganisms including free-living N_2-fixing bacteria, general plant growth promoting rhizobacteria and exerting biological control against plant pathogens. Thus, effect of interactions among the different soil microbes involved with antagonism or synergism when co-inoculated is a crucial step in the development of a revegetation strategy (Requena *et al*., 1997). Rhizobia and arbuscular mycorrhiza often interact synergistically, resulting in better roots nodulation, nutrient uptake and plant yield. In soils with low P content, this interaction is marked, especially with added phosphate. Such beneficial interactions have been shown in the following legumes: *Stylosanthes guyanensis*, *Centrosema pubescens*, *Medicago sativa*, *Phaseolus* sp., *Glycine max*, *Arachis hypogea*, *Vigna unguiculata*, *Pueraria* sp., *Trifolium repens* and *Trifolium subterraneum* (Microbiologyprocedure, 2011). The legume-rhizobium symbiosis is strongly influenced by AMF and there is some evidence to suggest that legume nodules contain AMF communities quite distinct from those found in the roots of legumes (Scheublin *et al*., 2004).

The rhizobium symbiosis is dependent on high concentrations of P and so the enhanced P nutrition arising from the AMF colonization can result in an increase in nodulation and N_2 fixation (Ganty *et al*., 1985). Kohler *et al*. (2007) evaluated the interaction between the inoculation with AMF and rhizobacterium in lettuce.

Rhizosphere soil from all microbial inoculation had significantly increased the dehydrogenase, urease, protease phosphatase activities and P, N content. Boby *et al*. (2008) studied the interaction between AMF and six soil yeasts on growth and nutrient of cowpea. The result showed that all yeasts had a synergistic interaction with AMF by improving plant growth, nitrogen and phosphorus uptake compared to single inoculation with *Glomus mosseae* alone.