CHAPTER 6

General discussion

The effects of AMF on the other microorganisms in the rhizosphere have been ascribed to direct interaction such as competition for inorganic nutrients between microorganisms (Christensen and Jakobson, 1993) or to indirect influence on the quality and quantity of the plant root exudates (Garbaye, 1991). Generally, AMF have a significant positive effect on host plant development, i.e., plant growth, biomass and nutrient uptake (Gutiérrez-Miceli *et al*., 2008). Furthermore, biological nitrogen fixation (BNF) by associative EDB has been isolated from plant tissue. It has several beneficial effects on host plant, such as plant growth stimulation, nitrogen fixation and indole-3-acetic acid (IAA) production (Will and Sylvia, 1990). Thus, this research was aimed to select, test, identify and evaluate the effects of AMF and EDB on growth of *C. alismatifolia* Gagnep. by morphological and molecular biological studies, efficiency of AMF, N_2 fixing potential and IAA synthesis of EDB and also examine potential as growth promoters and the effect of selected AMF mixed with EDB on growth and development.

C. alismatifolia is a non-legume plant. This research is the first report about EDB for N_2-fixation and IAA synthesis, in addition, the beneficial effect of AMF as growth promoter has also been revealed. Four isolates of EDB, i.e., ECL101, ECS202, ECS203 and ECS204 are beneficial microorganisms as N_2-fixing bacteria (ECS203 and ECS204) and IAA producer (ECS202 and ECL101) in this plant species. Application of ECS203 to *C. alismatifolia* plantlets could stimulate plant height, plant weight, diameter of rhizome, total leaf areas, chlorophyll content, N concentration and rhizome yield. The result from Chapter 4 showed that these isolates could be kept in 40% glycerol at-20°C for 3 months. Dong *et al*. (1995) reported that EDB isolated from sugarcane could be stored in 10% glycerol in a liquid N_2 storage apparatus for 2 months. Bacteria in cotton were maintained for long-term storage at -80 °C in 25 % glycerol (Chen *et al*., 1995). Four *Bacillus* sp. isolated from sugar beet cultures were stored at -80 °C in Tryptic Soy Broth (Difco) with 20% glycerol (Melnick *et al*., 2008). *Pseudomonas* sp. and *Bacillus* sp. isolated from olive tree were stored in 30% glycerol at -20 °C (Krid *et al*., 2010).

Long-term storage period of EDB will be beneficial for inoculum production. The research on the inoculum production of EDB had been involved in inoculum type, inoculum size and incubation time. However, inconsistency and variability in yield responses have been attributed to adverse conditions, such as interaction of rhizospheric organisms (Lucy *et al*., 2004), physical and chemical conditions of the soil (e.g., low pH), poor ability of the PGPR strain to colonize the plant roots, environmental factors, including high mean temperatures, and, low rainfall during the growing season (Broek *et al*., 2000) and to host cultivars (Vessey, 2003). Bio-fertilizer composition was found to promote growth of orchid plants by using

Rhizoctonia sp. Furthermore, it decreased the occurrence of root disease to prevent the usage of pesticide, and increased the beneficial components toward human health of medicinal orchids (Chang *et al*., 2008). Microbial N_2 fixation can increase N availability in plants. Total plant nitrogen due to N_2 fixation by EDB increased in shoot of the plant by *Azotobacter* sp. (Will and Sylvia, 1990). It was also found that *Williopsis saturnus* increased the dry weight and lengths of roots and shoots in maize (Nassar and El-Tarabily, 2005), *Pseudomonas* inoculants significantly increased root dry weight in spring wheat (Walley and Germida, 1997). *Sphingomonas* sp. isolated from potato increased 66% of shoot wet weight and 55% of root wet weight (Sturz *et al*., 1998). In this study, identification of 16S rDNA gene sequences of ECS203 showed 99.4% similarity to *Bacillus drentensis*. Many workers reported that *Bacillus drentensis* has activities as biocontrol agents of various fungi in black pepper (Aravind *et al*., 2009) and soybean (Senthilkumar *et al*., 2009), as phosphorous solubilizers in rice (Boro *et al*., 2004) and as auxin producers (Park *et al*., 2005).

AMF by soil sampling from *C. alismatifolia* rhizosphere at five locations have three types. The isolate No. 3 from Saithong national park location gave the best results in plant height, leaf areas, fresh weight, dry weight and P concentration in root and leaves. The infection percentage of isolate No. 3 was 70.65%. Comparing with the other species, it was lower than *C. longa* of which percentage of root infection ranged between 62 – 86 (Sumathi *et al*., 2008) and lower than *Glomus caledonium* in green-leaf and red-leaf lettuce with 90% and 97% infection, respectively, but it was higher than *Gigaspora margarita* in green-leaf and red-leaf lettuce with 19.5% and 55.8% infection, respectively (Saouy, 2008). Spores of AMF collected from rhizosphere of *C. alismatifolia* were propagated in sterilized sand with *Zea mays* as host plants. The results indicated that only isolate No. 3 could be propagated and produced enough quantity of spores. The identification of this AMF by 18S rDNA sequence revealed that isolate No. 3 was similar to *Glomus claroideum*, showing 97% homology. Many AMF species in the rhizosphere soil of plants with underground storage organs from ecologically-important regions could provide nutrients, thereby improving soil structure while maintaining soil fertility (Prasad *et al*., 1999). AMF have significant positive effect on maize development , higher uptake of N, P, Mg and Zn and also greater shoot masses (Gutiérrez-Miceli *et al*. 2008). Similar to Amerian *et al*. (2001) who reported that AMF increased P concentration in plant, i.e., roots, leaves and flowers. It has been reported that *G. intraradices* significantly increased concentrations of N, P, K, Mg, Mn and Zn under drought conditions compared to non-AMF plant.

Spore propagation is important for inoculum fertilizer. The inoculum fertilizer has been used for many agricultural crops, such as inoculum containing spores of *Glomus* sp. and *Gigaspora* sp. Mycorrhization of groundnut plants led to increase in a yield of up to 62.8% for dry pods of healthy plants compared to uninoculated plants as control. Moreover, the mycorrhizal symbiosis with groundnut roots increased the resistance of plants to diseases, and positively influenced the physiology of groundnut plants (Zachée *et al*., 2008). Similarly, the inoculum by *Scutellospora calospora* significantly increased nutrient uptake and plant growth of cocoa seeding. The dry matter yield and the tissue N and K concentrations in the plant increased significantly compared with control (Chulan, 1991). Yeasmin *et al*. (2007) reported that after using AMF inoculum, the soil nutrients, as well as, root colonization for rice plants were greatly affected. Soil nutrients and percentage of rice roots colonization were increased. AMF greatly improved the soil nutrients such as nitrogen and phosphorus, as well as, growth of rice plants. Many researches were conducted in biofertilizer application to various plant species. The success of biofertilizer application involved many factors, such as soil characteristics, strains of inoculates, physiological state of microorganism. This great variability and inconsistency have been observed in the application of mineral fertilizers in diverse crops cultivated under different environmental and soil conditions, and the yield response to mineral fertilizers has been recorded. The experiments were carried out in the same or different sites and on the same crops, and in different years and varying environmental conditions (Fuentrs-Ramirez and Caballero-Mellado, 2005). In the present research, chemical fertilizer application was combined with microorganism (AMF and EDB). The result indicated that it could affect growth of *C. alismatifolia* including plant height, plant weight, leaf area, nutrient concentration and IAA concentration. Similar reports were also revealed in other non-legume plants. Gutiérrez-Miceli *et al*. (2008) studied the effect of *Glomus claroideum, G. fasciculatum* and native diazotrophic bacteria on maize cultivation. The result showed that these could improve plant height, number of leaves and stem diameter. Furthermore, interaction between rhizosphere bacteria and AMF was also found which could enhance plant growth, such as total root length in sea oats (Will and Sylvia, 1990), promote root growth and increase shoot height and shoot weight in wheat (Chanway *et al*., 1988). At flowering stage (at 12 WAP), chemical fertilizer combined with AMF and EDB gave better quality of inflorescence than non-fertilizer plus AMF only. Chemical fertilizer plus AMF+ECS203 gave the high average result in inflorescence quality, fresh and dry weight of plant and N, P, K concentrations. Similar result also was found in inoculation with vesicular-arbuscular mycorrhizal fungi and rhizobacteria in harlequin plant. The number of inflorescence and the number of flower per inflorescence were increased by inoculation treatments (Scagel, 2004). At harvest stage, rhizome quality of *C. alismatifolia* had the best value when supplied with chemical fertilizer plus AMF+EDB. This is similar to Will and Sylvia (1990) who revealed that fertilizer N could increase root and shoot dry mass of sea oats and there was an interaction between rhizosphere bacteria and AMF.

The biofertilizer must be verified to have adequate inoculum potential so that it can multiply in the rhizosphere sufficiently to have a beneficial effect (Kennedy *et al*., 2004). For this reason, AMF and EDB were studied for their efficiency as biofertilizer. In this study, fertilizer application had effect on growth and development of *C. alismatifolia* more than non-fertilizer. Moreover, the application of AMF and EDB in cooperation with chemical fertilizer could improve the efficiency of chemical fertilizer utilization and reduce the chemical fertilizer requirement of this plant. The inoculated plant with AMF+ECS203 had the best result compared with the others. It may be caused by efficiency of ECS203 for N_2 fixation (the result in Chapter 4). Furthermore, AMF have potential to increase the absorbing surface area of the roots, i.e., water and plant nutrient, especially P. Therefore, the best AMF in Chapter 3 plus ECS203 could be used to reduce chemical fertilizer utilization in *C. alismatifolia*. However, the further experiment should be explored to find out the optimum rate of fertilizer application when applied with biofertilizer treatment. The inoculum production from AMF (*Glomus claroideum*)+ECS203 (*Bacillus drentensis*) also should be further studied in the future.