CHAPTER V

DISCUSSION

From field observation, soybean plants cultivated in the farmers' fields at Chamkaar Andong and Chamkaar Leu could form nodules even the site where soybean was cultivated in the first year. Since all fields have not been inoculated, nodule formation indicating the occurrence of native of root nodule bacteria which are able to infect soybean host plants. The data from farmers' interview indicated that after the old rubber trees were cut down and the areas between the rows of the new rubber tree transplanted seedlings were used for soybean cultivation, the yield of soybean in subsequent years decreased. The interviewed farmers thought that reduction of plant nutrients in the soil was the factor involved with decrease of soybean seed yield in the following year after the rubber trees were cut down. However, soil analysis data showed that there were no differences of soil pH, available P, exchangeable K, and soil organic matter among the soils with different soybean cultivated periods suggesting that soil quality might not be yield limiting factor but shading by the grown up rubber trees seemed to be the possible factor involving with yield reduction of soybean. Existing of native root nodule bacteria even in the site where soybean was cultivated for the first year supporting that soybean cultivated in the rubber tree plantation could fix atmospheric nitrogen.

According to Somassgaran and Hoben (1994) short turn trials with Leonard's jars or sterile sand culture pots can provide adequate basis for gross comparison of root nodule bacterial strains.

From this research, a variation among different Cambodian root nodule bacterial isolates from different soybean trap hosts on the infectiveness abilities and N_2 fixation were observed from the control room experiment which supported Somasegaran and Hoben (1994). Thompson *et al.*, (1991) isolated over 1,500 root nodule bacteria from uninoculated traditional soybean growing areas of northern Thailand and reported that most isolates were slow-growing *Bradyrhizobium japonicum*. In this research, half of the total collected native isolates of root nodule bacterial isolates were fast growing and acid producing bacteria. Fast-growing isolates from soybean nodules were identified as *Sinorhizobium fredii* (Scholla and Elkan, 1984 cited by Berkum and Eardly, 1998). It may be possible that the fast growing native soybean root nodule bacteria from Cambodia belong to the same group as *S.fredii*. Further study is needed to confirm the correct classification.

For pot trial, the autoclaved soil was used for cultivation of DT 84 soybean under outdoor condition using tap water for irrigation. However soybean plants from uninoculated control and single EA inoculated treatments could form root nodules suggesting there was contamination of root nodules bacteria from surrounding environment. Among the four Cambodian root nodule bacteria used for pot trial, CD_1YD8 showed the best performance to improve N uptake of the whole plant at 30 days after inoculation under controlled room condition with N free nutrient solution. E-value of CD_1YD8 was 86% while CL_3B1 and CL_4HK7 were moderately effective with E-values of 55 and 45% respectively and CD_2P was the poorest with E-value of 23%. However under pot trial using autoclaved soil as the growth medium, CD_2P and CL_4HK7 were not different significantly from CD_1YD8 for the effects on SDW, RDW, NDW and shoot N uptake at V_6 stage which was about 32 DAS. Furthermore

at this growth stage CD_2P and CL_4HK7 were the only two root nodule bacterial isolates which could increase significantly SDW, RDW and total plant dry weight over uninoculated control treatment. On yeast mannitol agar, CD_1YD8, CD_2P, CL_3B1 and CL_4HK7 could form single colony within 2 days thus there might be no differences among these root nodule bacterial isolates for their growth rate after inoculation into the soil. Thus no differences of nodule dry weight at V_6 stage were not observed among Cambodian root nodule bacterial inoculated treatments and all treatments improved NDW significantly compared to uninoculated control. This experimental result suggested that there were no differences of infectiveness among these four Cambodian root nodule bacterial isolates. However, CD_2P and CL_4HK7 were better than CL_3B1 and CD_1YD8 for the effective on SDW and RDW improvement of DT 84 soybean at V_6 stage but these isolates had no effect on shoot N uptake as compared to uninoculated control. The abilities of rhizobial and Bradyrhizobial isolates to produce plant hormone have been reported Antoun *et al.* (1998). It might be possible that some of these Cambodian root nodule rhizobial isolates could produce phytohormone resulting in improvement of root and shoot biomass of soybean at V_6 stage. The population of CD_2P and CL_4HK7 in the soil after inoculation might be more than those of CL_3B1 and CD_1YD8 resulting in higher production of phytohormone which subsequently affecting root and shoot development. Single inoculation of Th7 bradyrhizobial strain did not have any significant effects on all studied parameters of soybean plant at V_6 stage except one case on RDW which increased significantly compared to uninoculated control. Since Th7 is slow growing root nodule bacterial strain, the population of Th7 in the soil at 32 days after seed sowing might not be as high as fast growing root nodule bacterial

from Cambodia. At this growth stage, the performance of Th7 strain was therefore rather poor. Endophytic actnomycetes (EA) has been recognized as one of the microorganisms being able to produce growth regulators of other organisms (Matsukuma *et al.*, 1994 and Okasaki *et al.*, 1995). The results from pot trial indicated also that single inoculation of EA could increase significantly SDW, RDW, TDW and N uptake of shoot of DT 84 soybean at V_6 growth stage. The beneficial effects of EA in this experiment on soybean growth and N uptake of shoot might be due to phytohormone production of EA. When the effects of dual inoculation was compared with single inoculated one, the experimental results showed that there was no differences between single inoculation and dual inoculation of EA and root nodule bacterium except one case (EA + CD_2P) which significant synergistic effects of EA + CD_2P on RDW, NDW and total plant dry weight were observed. The synergistic effect of EA + CD_2P indicated that EA could compatible with CD_2P at this growth stage.

At $R_{3.5}$ stage, Th7 bradyrhizobial strain showed its better performance. The effectiveness of this bradyrhizobial strain was not different from most of the tested Cambodian root nodule bacterial isolates based on the effects on SDW, RDW, NDW, N uptake of shoot and N_2 fixing ability. Anyhow, the percentage of seasonal of fixed N of Th7 inoculated soybean was lower than that of CD_1YD8 inoculated one which was the best treatment for improvement of the amount and percentage of seasonal fixed N. At $R_{3.5}$ stage CD_1YD8 nodule dry weight of CD_2P inoculated soybean plant was not different significantly from that of uninoculated control one while the rest of root nodule bacteria including Th7 improved significant nodule dry weight. The decline of effectiveness CD_2P root nodule bacterial isolate at $R_{3.5}$ stage might be due

to more nodule degradation of CD_2P inoculated soybean plant than those from the other single root nodule bacterial inoculated treatments. Nevertheless the effectiveness of CD_2P was statistically not different from those of CL_4HK7, CL_3B1 and Th7 as considering from SDW, RDW, NDW, amount and percentage of seasonal fixed N at $R_{3.5}$ stage including seed yield of DT 84 soybean. The performance of CD_2P root nodule bacterial isolate to support DT 84 soybean host plant under pot trial with soil as growth medium was different from that under control room condition with sterile sand and N free nutrient solution. Under pot trial CD_2P was still effective to increase significantly growth of shoot and root, N_2 fixing ability and seed yield over the control but this isolate was ineffective under control room condition. Pueppke (1986) reported that nodulation of Mecall and Vicoja soybean in growth pouches was very different to that in an autoclaved greenhouse soil mixture. This author proposed that the soil environment appeared to obliterate strain cultivar-specific variation that is expressed when inoculated plants were grown hydroponicaly is growth pouches. The different performance of CD_2P which depended on cultivated condition agreed with the report of Pueppke (1986). In soil which was more fertile than sand the inoculated root nodule bacteria particular CD_2P might be able to survive better and could express its effectiveness more clearly. However, from the experimental result in the control room, CD_1YD8 was selected as the highly effective isolate. Under pot trial with soil as the growth medium, the effectiveness of CD_1YD8 was also confirmed as the best root nodule bacterial inoculated treatment for improvement of N_2 fixation and seed yield of DT 84 soybean.

At $R_{3.5}$ stage, DT 84 soybean still responded well to single EA inoculation regarding to shoot and root development. In this treatment nodule formed by

contaminated root nodule bacteria could fix more N than those from uninoculated plants. At harvest, soybean plant from this treatment provided significant more seed yield than the control. Such good performance of EA inoculated soybean indicated that this EA could compatible well with DT 84 soybean. Since antagonistic ability of this EA against some fungal diseases has been reported (Thapanapongworakul, 2003) it is worth while to study about the effect of EA inoculation on disease infection in the future. If this EA could be used as biocontrol agent for soybean also its usefulness for soybean cultivation in Cambodia is very attractive.

When the compatibility of each nodule bacterial isolate or strain with DT 84 soybean was considered from its effects on growth, nodulation and N_2 fixation of this soybean variety, the experimental results indicated that all of the tested root nodule bacteria could compatible well with this soybean host plant if single inoculations were used. In this study, significant correlation among the following studied parameters at $R_{3.5}$ stage, NDW, SDW, N uptake of shoot, amount and percentage of fixed N including seed yield. Zary *et al.,* (1978) and Wadisinisak and Weaver (1985) reported the total nodule mass formed by effective root nodule bacteria and quantity of N fixed was linearly related the experimental results of this study supported the report of Zary *et al.,* (1978) and Wadisinisak and Weaver (1985). However when seed yield was considered CL_4HK7, and Th7 were not be able to increase significantly seed yield over that of uninoculated control. Nevertheless seed yield of CL_4HK7 and Th7 inoculated soybean plants inoculated were about 79 and 84% higher than that of the control treatment.

The performances of soybean plants from single Th7 and CL_3B1 inoculated treatments after $R_{3.5}$ stage seemed to drop down as compared to those at $R_{3.5}$ stage

resulting in low seed yield. Plant performance declining of these treatments were not due to treatment effect because the soybean's shoot showed symptom of disease infection as shown in Fig 4.

Regarding to the effects of dual inoculated treatments in which significant synergistic effects EA + CD_2P, on some studied parameters at V_6 and $R_{3.5}$ and percentage of seasonal fixed N, no. of pods per plant and seed yield by EA + Th7 while depressive effects of EA + CD_1YD8 on N_2 fixation, no. of pods per plant and seed yield were observed. Tokala *et al.* (2002) studied the effect of *streptomyces lydicus* WYEC 108, endoptytic actinomycetes strain on nodulated pea plant.

Fig. 4: Symptom of plant disease at soybean's shoot

They reported that *S. lydicus* WYEC 108 could colonized pea root resulting in increasing of root nodulation frequency, possibly at the level of infection by

Rhizobium spp. S.lydicus also colonized and sporulated within the surface cell layers of the nodules. Colonization of *S. lydicus* improved the size of nodule and improved the vigor of bacterial within the nodules by enhancing nodules assimilation of iron and possibly other soil nutrients. None of these mechanisms were tested in this research but these mechanisms could be occurred in the case that synergistic effect of dual inoculation EA and each of tested root nodule bacterial isolates or strain were observed. The responses of DT 84 soybean to EA + Th7 and EA + CD_2P agreed with the report of Thapanapongworakul (2003). Samac *et al.* (2003) studied the effects of antibiotics producing streptomyces on nodulation of alfalfa and found out that eight of the total of 15 *Streptomyces* strains inhibited invitro growth of five or more of *Sinorhizobium meliloti* strains while four *Streptomyces* strains had no effect on growth of any tested strains. In growth chamber assay, two of six *Streptomyces* strains, when inoculated into the planting mix, significantly reduce plant dry weight compared to treatment with *S.meliloti* alone, but did not reduce the numbers of nodule. The other strains with strong in vitro antibiosis activity did not affect plant weight significant. They suggested that careful selection of *Streptomyces* isolate for use in biological negative impacts on *Rhizobia*. The experimental results of this study supported suggestion of Samac *et al.* (2003).

In this study, the significant depressive effects of EA in dual inoculated with CD_1YD8 on N_2 fixation number of pods per plant and seed yield were observed suggesting that EA could not be compatible well with this root nodule isolate. At $R_{3.5}$ and the later growth stages, soybean host plant might have more requirement of carbonsynthate including nutrients for seed yield than the earlier growth stage. Samac *et al.* (2003) proposed that if the leguminous host plants or rhizophere soil of the

plants provide sufficient nutrients to support development of endophytic *streptomyces*, the occurance of endophytic actinomycetes will not affect plant biomass accumulation. In this study, depressive effect of EA in dual inoculation with CD_1YD8 on N_2 fixation and seed yield might be due to competition of photosynthate and nutrients provided by the host plant between EA and CD_1YD8.

Significant variation of effectiveness among root nodule bacterial strain has been reported by several authors (Hobbs and Mahon, 1982; Bremer *et al.*, 1990; Rosas *et al.*, 1998). In this study, the responsive pattern of DT 84 soybean to bradyrhizobium or root nodule bacterial isolates also varied with strain or bacterial isolates which supported the reports of Hobbs and Mahon, 1982; Bremer *et al.* 1990; and Rosas *et al.* 1998.

The experimental results of pot experiment from this study should be confirmed by field experiment in the future in order to ensure that the effective Cambodian root nodule bacterial used in this study can perform well under natural environment particularly soybean cultivated field in Cambodia. If their performances under fields testing are also impressive, these native isolates can be used for inoculant production in Cambodia in the future. Nevertheless additional research on competitiveness of these native root nodule isolates under Cambodian soil environment including the effect of soil management on population of indigenous root nodule bacteria for soybean are also essential.