Effects of Selected Endophytic Actinomycetes (*Streptomyces* sp.) and Bradyrhizobia from Myanmar on Growth, Nodulation, Nitrogen Fixation and Yield of Different Soybean Varieties

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ABSTRACT

Effects of endophytic actinomycetes and Myanmar Bradyrhizobia on growth, nodulation, nitrogen fixation and seed yield of three recommended soybeans from Myanmar (Hinthada), Thailand (SJ 5) and Cambodia (DT 84) were studied in pot experiments at Chiang Mai University during November, 2008 to March 2009. Sterile soil by autoclaving was used for cultivation under open field using tap water. In each pot trial, one soybean variety was tested, using randomized complete block experimental design with 3 replications and 6 treatments. The tested treatments were as follows: uninoculated control treatment, two Bradyrhizobial inoculated treatments with MA and MB, Bradyrhizobial isolates from Myanmar, single inoculated treatment with a selected endophytic actinomycetes, EA (Streptomyces sp.) and two dual inoculated treatments with EA+MA and EA+MB. N2 fixations of soybean plants were evaluated by ureide technique, using root bleeding sap. We found out that the tested treatments had no significant effects on nodule dry weight of the tested soybean varieties at both V6 and R3.5 growth stages. Single inoculation with MB resulted in significant improvement of shoot N uptake at R3.5 stage, amount of seasonal fixed N and seed yield of all tested soybean varieties compared to uninoculated control but MA was effective only for Cambodian soybean. Single inoculation with EA had no significant effects on all studied parameters and dual inoculation with EA+MB even showed significant depressive effect on amount of seasonal fixed N of all soybean varieties compared with single inoculation with MB treatment. The effects of dual inoculation with EA and MA on all studied parameters were not significant but this treatment tended to be more effective than single inoculation with MA.

Key words: Bradyrhizobium, Endophytic actinomycetes, Nitrogen fixation, Nodulation, Soybean

INTRODUCTION

In Myanmar, soybean is one of the important cash crops to the increasing demand for domestic consumption and export (CSO, 2006). Urea is the main source of nitrogen applied to all crops grown in Myanmar but it is very expensive and not readily available (Hla and Han, 1988). Root nodule bacterial inoculant can be used to substitute the nitrogenous fertilizers in food leguminous crops. At present, 250,000 packages of peat-based root nodule bacterial inoculants for seven leguminous crops including soybean are produced annually by Plant Pathology Section, Department of Agricultural Research (DAR) and distributed through Extension Division of Myanmar Agriculture Service (MAS) (Maw et al., 2006). Exotic Bradyrhizobial strains as follows: TAL 377, TAL 379 and TAL 102 from NifTAL Project are used in root nodule bacterial inoculant production for soybean (DAR, 2004).

Recently, Thi (2007) collected 38 native Bradyrhizobial isolates from upper Myanmar soybean growing area and five effective isolates were screened out based on their infectiveness and the abilities to improve shoot dry weight of soybean plants at 45 days after sowing under bottle jar with sterile sand and N free medium testing. However, further study on the effects of native bradyrhizobial isolates from Myanmar on N2 fixation and seed yield of soybean is needed in order to guarantee the potential of these indigenous microbes for soybean production. Not only bradyrhizobial inoculation is important for soybean cultivation by poor Myanmar farmers as valuable cheap source of nitrogen enough N2 fixation but plant disease control by biological means are also necessary and attractive topic for investigation.

Yuan and Crawford (1995) reported that several new or rare species of actinomycetes were discovered from plants and their secondary metabolites might be promising sources of novel antibiotics and growth regulators of other organisms. Control of soil-borne diseases with endophytic actinomycetes biocontrol agents has elicited considerable interest (Paulitz and Linderman, 1991).

In case of soybean, inoculation of selected endophytic actinomycetes (*Streptomyces sp.*) isolated from sweet pea which showed antagonistic ability against fungal plant diseases could infect and improve nitrogen uptake of the soybean plant grown under controlled condition about 83% compared to uninoculation control treatment and such endophyte could be compatible well with *Bradyrhizobium* (Thapanapongworakul, 2003).

It is worthwhile to investigate further whether this selected endophytic actinomycetes can be compatible with native bradyrhizobial isolates from Myanmar because the research result can be used as the guide line to improve the growth and yield of soybean by biological means. Moreover, selection of beneficial microbes which can be compatible with a wide range of soybean varieties is also important for agronomic application point of view.

Thus the research was therefore conducted with the objective to evaluate the responses of soybean varieties from different origins to selected endophytic actinomycetes and Bradyrhizobial isolates from Myanmar in terms of plant growth, nodulation, nitrogen fixation and seed yield.

MATERIALS AND METHODS

Three pot experiments were conducted outdoor at Faculty of Agriculture, Chiang Mai University, Thailand during November 2008 to March 2009. The soil collected from Mae Hia Research and Training Center, Faculty of Agriculture, Chiang Mai University was sterilized by autoclaving at 125°C for 1 hour before using for soybean cultivation in order to reduce the native population of root nodule bacteria in the soil. The autoclaved soil had pH 7.6, 55 mg/kg of NH_4^+N, 15 mg/kg of NO_3^-N, 40 mg/kg of available P, 87 mg/kg of exchangeable K, 1447 mg/kg of exchangeable Ca and 101 mg/kg of exchangeable Mg. One of these three recommended soybean varieties from Myanmar (Hinthada), Thailand (SJ 5) and Cambodia (DT 84) was grown in each pot experiment, using randomized complete block design (RCB) with 3 replications and 6 treatments. There were three plants per pot containing 10 kg of sterile soil. The treatments for each soybean variety were uninoculated control treatment, single inoculation with selected endophytic actinomycetes (EA), two single inoculated treatments with MA and MB, native Bradyrhizobial isolates from Myanmar soil and two dual inoculated treatments with EA+MA and EA+MB. MA and MB were isolated from nodules of Myanmar soybean trap host grown in soil sample collected from soybean cultivated area in Bagan, Middle Myanmar. This soybean trap host was a local variety used by owner of the selected soybean cultivated field. These two native Bradyrhizobial isolates could improve shoot dry weight of Myanmar local soybean variety about 50-56% over that of uninoculated control under controlled room condition with sterile sand and N-free nutrient solution (Soe, unpublished data). From preliminary study of MA and MB root nodule bacterial isolates, single colony of MA and MB isolates could be observed after streaking on yeast manitol congo red agar for five days. They did not absorb congo red dye and showed typical colony morphology of root nodule bacteria. On YMA with Bromothymol blue dye, these two isolates changed slightly the color of this medium from green to blue. Both of them had rod shaped and were Gram negative bacteria. According to Vincent (1972) both MA and MB could be identified as the bacterial isolates in genus *Bradyrhizobia.*

However in this study, identification of these two isolates into species or strains were not possible because the detail on IAA production or serological differences or information on their genetics are required which were not inoculated in this scope of this research. These two Bradyrhizobial isolates were tested for their infectiveness and effectiveness with the same soybean trap host under controlled room condition with sterile sand and N-free nutrient solution (Somasegaran and Hoben, 1994). Under such testing condition, both MA and MB Bradyrhizobial isolates could improve shoot dry weight of the local Myanmar soybean test host about 50-56% over that of uninoculated control at one month after inoculation. The selected endophytic actinomycetes was identified as *Streptomyces* sp. and was tested previously as the effective endophyte which could be compatible with USDA 110 strain of *Bradyrhizobium* for one of Thai soybean without detail of variety (Thapanapongworakul, 2003).

EA and Bradyrhizobial isolates were grown in Inhibited mold agar 2 (IMA

2) and yeast mannitol broth, respectively. After the maximum density of mycelia of EA or cell suspension of each *Bradyrhizobium* were obtained (>10^9 cfu/ ml), fresh liquid culture of each microbe was inoculated to the seeds of each soybean variety germinated in plastic seedling tray, using sterile peat-moss substrate. The rate of inoculation was 10^6 cfu/seed for both EA and bradyrhizobial isolates. Ten days after seed germination, soybean seedlings for all treatments were transplanted into the soil using three plants per pot with 10 kg of soil.

There were three sets of pots with complete treatments and replications, one set for each harvest at V6, R3.5 and maturity stage. Ordinary tap water was used for irrigation once a day through out the growing season. Nodule dry weight, root dry weight and shoot dry weight at V6 and R3.5 growth stages, relative ureide index (RUI) of root bleeding sap and total N uptake of shoot at R3.5 stage, percentage (Herridge and Peoples, 2002) and amount of N derived from N_2 fixation throughout the growing season and seed yield were collected and statistically analyzed by F-test.

The root bleeding sap samples were analyzed for amino-N (Yemm and Cooking, 1955), NO_3^-N (Cataldo et al., 1975) and ureide-N (Young and Conway, 1942). The total N content of the plant samples were analyzed by the method suggested by Novozamsky et al. (1974).

Relative ureide index (RUI) of root bleeding sap was calculated according to Peoples et al. (1988).

$$\text{Relative ureide index (\%)} = \frac{4 \text{ x ureide}^*}{(4 \text{ x ureide}^* + \text{amino acid}^* + \text{nitrate}^*)} \text{ x } 100$$

Note: (* = mole)

Percentage of fixed N throughout the growing season of soybean was calculated by the equation proposed by Herridge and Peoples (2002).

y = 4.8 + 0.83x (at early pod filling stage on R3.5 stage);

where: y = relative ureide index (%)

x = nitrogen derived from air (%)

The total N accumulation or N uptake of the shoot was calculated according to this formula:

$$\text{N uptake (g) of plant} = \frac{\text{\% N (g) x Dry Wt. (g)}}{100}$$

RESULTS

At V6 and R3.5 stages, Hinthada soybean plants from uninoculated control treatment produced root nodule about 0.17 and 0.26 g dry weight per plant while those from single inoculation with EA treatment had dry weight of root nodule about 0.19 and 0.29 g/plant respectively. Inoculation of MA or MB either alone or incombination with EA did not have significant effects on root nodule dry weight compared to uninoculated control treatment at both growth stages. However the plant inoculated with MA and MB Bradyrhizobial isolates had more nodule dry weight than the control plants about 23 to 65% at R3.5 stage and about 54 to 46% from those with dual inoculation (Figure 1).

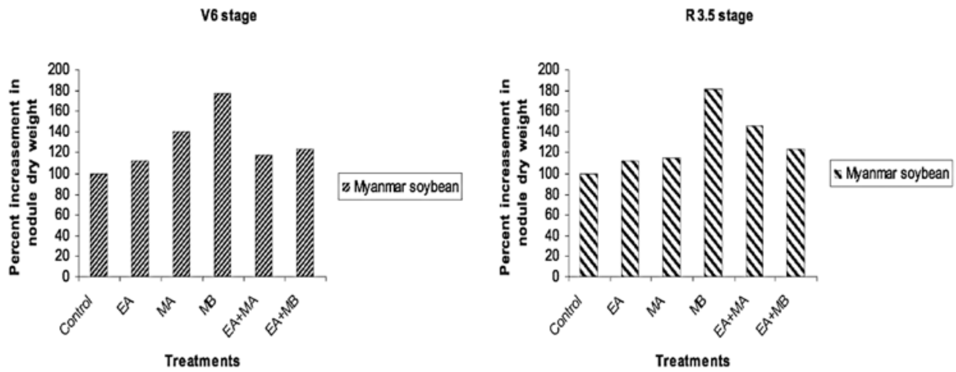

Figure 1. The percent increase in nodule dry weight of Hinthada soybean variety inoculated singly with EA, MA, MB and coinoculation of EA and MA or MB at V6 and R3.5 stages.

In Hinthada soybean, the tested treatments had significant effects on shoot N uptake at R3.5 growth stage, the amount of total fixed N and seed dry weight only. Single inoculation of EA and MA were not effective to improve the studied parameters mentioned above while single inoculation of MB increased shoot N uptake (238%), amount of fixed N throughout the growing season (456%) and seed yield (96%) ($P<0.005$) compared to the uninoculated control treatment (Table1).

Table 1. Effects (1) of selected endophytic actinomycetes and bradyrhizobial isolates on shoot N uptake (SN) at R3.5 stage, percentage and amount of fixed N throughout the growing season and seed yield of Hinthada soybean variety.

Treatment	SN (mg N/plant)	fixed N		seed yield (g/plant)
		Percentage (%)	mg N/plant	
Control	152b* (2) (100) (3)	61.46^{ns} (100)	92.33c** (100)	9.84c* (100)
EA	177b (116)	67.43 (110)	119.33bc (129)	11.49c (117)
MA	180b (118)	80.77 (131)	140.67bc (152)	12.52bc (127)
MB	513a (338)	99.93 (163)	513.00a (556)	19.33a (196)
EA+MA	227b (149)	91.90 (150)	151.60bc (164)	14.07abc (143)
EA+MB	340ab (224)	86.23 (140)	294.00b (318)	17.08ab (174)
CV%	42.08	17.60	48.03	21.77

(1) means of 3 replications
(2) means within a column followed by the same letter are not significantly different (LSD, $P>0.05$)
(3) values in parenthesis are comparative values compared with the control

The effects of single inoculation of MA or MB and combined inoculated treatments (EA+MA, EA+MB) on shoot N uptake and seed yield of Hinthada soybean were not different from each other significantly. Depressive effect of dual inoculation of EA and MB was clearly shown in the amount of fixed N throughout the growing season compared to single inoculation of MB ($P<0.05$).

In SJ5 soybean variety, nodule formations in non Bradyrhizobial isolated treatments were also observed. Though bradyrhizobial inoculated treatments either alone or incombination with EA did not affect nodule dry weight of SJ5 soybean significantly at both V6 and R3.5 stages compared to uninoculated treatment but inoculated SJ5 soybean plants had more nodule dry weight about 3-18% than uninoculated one (Figure 2).

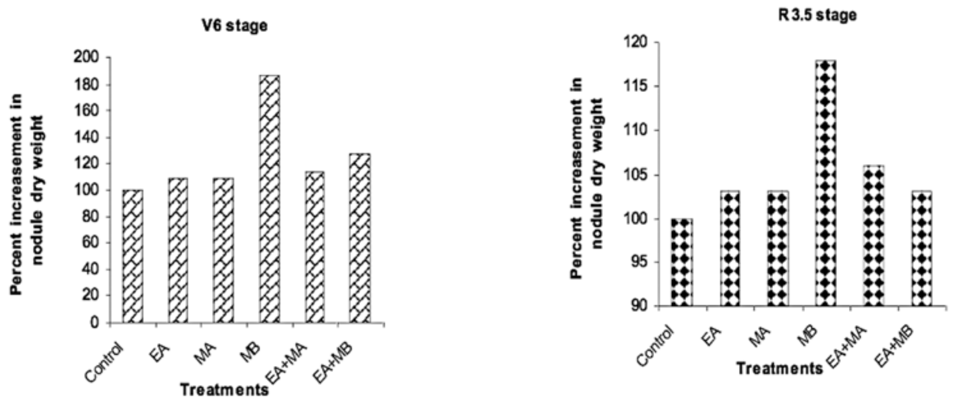

Figure 2. The percent increase in nodule dry weight of SJ5 soybean variety inoculated singly with EA, MA, MB and coinoculation of EA and MA or MB at V6 and R3.5 stages.

In SJ5 soybean, the tested treatments affected shoot dry weight significantly at V6 growth stage, root dry weight and shoot N uptake at R3.5 stage, amount of total fixed N and seed yield (Table 2). At V6 stage, shoot dry weight of SJ5 soybean increased significantly by single inoculation of MB (77%), MA (39%) and EA (33%) compared to uninoculated control treatment. Combined inoculation of EA and each of Bradyrhizobial isolates were less effective than single inoculation of *Bradyrhizobium* particularly EA+MB which depressive effect on shoot dry weight was significant. At R3.5 stage, single inoculation of MA and MB were effective to improve root dry weight of soybean about 61 and 72% compared to uninoculated control but only MB had positive effect on shoot N uptake.

Though the effects on measured (RUI) and calculated (%P) N_2 fixing parameters were not significant (data not shown), the tested treatments affected significantly the amount of total fixed N. The amount of total fixed N in single and dual inoculated soybean plants was within the range of 14-273% of that of uninoculated control. Single inoculation with MB was the most effective to increase significantly the amount of total fixed N of SJ5 soybean (273%) while the effects of MA and EA were not significant. Depressive effect by dual inoculation of EA and MB on amount of total fixed N was also observed compared with that of single inoculation of MB ($P<0.05$).

Table 2. Effects (1) of selected endophytic actinomycetes and bradyrhizobial isolates from Myanmar on shoot dry weight (SDW) at V6 growth stage, root dry weight (RDW) and N uptake of shoot (SN), amount of fixed N throughout the growing season (fixed N) and seed yield of SJ5 soybean variety.

Treatment	Growth Stages				
	V6	R3.5			
	SDW (g/plant)	RDW (g/plant)	SN (mg N/plant)	fixed N (mg N/plant)	seed yield (g/plant)
Control	3.76d**(2) (100)(3)	2.39 c* (100)	107c* (100)	63c** (100)	8.69b* (100)
EA	5.01bc (133)	2.53c (106)	120bc (112)	72c (114)	11.38ab(131)
MA	5.23bc (139)	3.84ab (161)	150bc (140)	112bc (178)	9.14b (105)
MB	6.64a (177)	4.10a (172)	280a (262)	235a (373)	15.74a (181)
EA+MA	4.12cd (110)	2.91bc (122)	167bc (156)	119bc (189)	12.55ab (144)
EA+MB	5.27b (140)	3.82ab (160)	200ab (187)	137b (218)	12.83ab (148)
CV%	12.52	19.31	27.62	28.68	19.93

(1) means of 3 replications
(2) means within a column followed by the same letter are not significantly difference (LSD, P<0.05)
(3) values in parenthesis are comparative value compared with the control

Regarding to the effect on seed yield, it was found that single inoculation with MB was the most effective to improve significantly seed yield of SJ5 soybean (81%) compared to uninoculated treatment. However, MB was not different significantly from the following treatments, EA, EA+MA and EA+MB. The last three inoculated treatments could increased seed yield of SJ5 soybean within the range of 31-48% over that of the control treatment but the differences of seed yield of these treatments and the control were not significant. MA was ineffective and seed yield improvement by this treatment was only 5% over that of the control but MA was not different significantly from the rest except MB.

In DT84 soybean variety, uninoculated control plant produced root nodule about 0.25g/plant at V6 stage and did not show further development of nodule dry weight at R3.5 stage. Singly inoculated EA plants had nodule dry weight as much as uninoculated control. No significant effects of tested treatments on nodule dry weight of DT84 were found even though MA and MB Bradyrhizobial inoculated singly plants had more nodule dry weight about 28% and 84% than inoculated control at V6 stage (Figure 3).

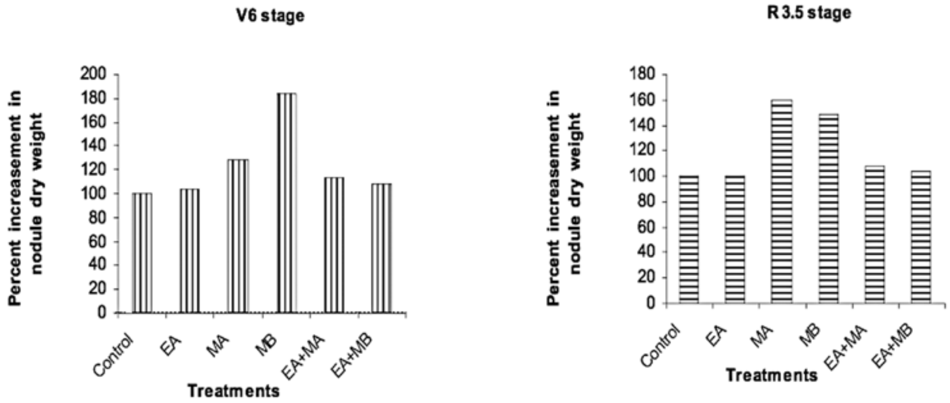

Figure 3. The percent increase in nodule dry weight of DT84 soybean variety inoculated singly with EA, MA, MB and coinoculation of EA and MA or MB at V6 and R3.5 stages.

In DT84 soybean variety, MA was more effective than MB at R3.5 stage even though MB performed better ($P<0.05$) at V6 stage for improvement of shoot dry weight (56% over that of the uninoculated control). Nevertheless, nodule dry weight of MB inoculated soybean plants at V6 and R3.5 growth stages were about 84 and 48 % higher than that of uninoculated control while those from MA inoculated ones were about 28 and 60%. At R3.5 stage, shoot dry weight of soybean plants from inoculated treatments were about 16-69% over that from uninoculated treatment however only the effects of single inoculation with MA was significant (Table 3).

Table 3. Effects (1) of selected endophytic actinomycetes and bradyrhizobial isolates from Myanmar on shoot dry weight (SDW) at V6 and R3.5 growth stages, relative ureide index (RUI) at R3.5 stage, percentage (%P) and amount of fixed N throughout the growing season (fixed N), N uptake of shoot (SN) and seed yield of DT84 soybean variety.

Treatment	Growth Stages						
	V6	R3.5					
	SDW (g/plant)	SDW (g/plant)	SN (mg N/plant)	RUI %	fixed N %	fixed N mg N/plant	seed yield (g/plant)
Control	5.01b$^{**(2)}$ (100)$^{(3)}$	7.35b* (100)	113c**(100)	48.33c* (100)	52.45c* (100)	65c** (100)	7.07c** (100)
EA	5.25b (105)	8.51b (116)	140bc (124)	50.33c (104)	54.86c (105)	81c (125)	8.41bc (119)
MA	5.50b (110)	12.39a (169)	437a (387)	87.74a (182)	99.93a (191)	437a (672)	10.97a (155)
MB	7.84a (156)	9.51b (129)	240b (212)	81.67ab (170)	92.61ab (177)	222b (342)	10.25a (145)
EA+MA	5.80b (116)	9.47b (129)	180bc (159)	70.00abc (145)	78.56abc (150)	135bc (208)	9.69a (137)
EA+MB	5.54b (111)	8.62b (117)	187bc (165)	66.33bc (137)	74.14bc (141)	111c (171)	9.56ab (135)
CV%	12.73	14.73	30.67	18.87	20.31	32.48	9.47

(1) means of 3 replications
(2) means within a column followed by the same letter are not significantly different (LSD, $P>0.05$)
(3) values in parenthesis are comparative values compared with the control

Not only shoot dry weight, MA was significantly more effective than MB for the effects on shoot N uptake at R3.5 stage. Single inoculation with MA resulted in improvement of 287% shoot N uptake while that of single inoculation with MB increased about 112% compared to uninoculated control. In DT84 soybean, single inoculation with EA did not show significant effect on all studied parameters. Dual inoculation of EA and the tested bradyrhizobial isolates produced depressive effect compared with single inoculation of each Bradyrhizobial isolate. Such depressive effects of dual inoculation on shoot dry weight, shoot N uptake and amount of total fixed N were clearly observed in EA+MA treatment while that of EA+MB was shown on the amount of total fixed N.

Regarding to N2 fixing of DT84 soybean, it was found both MA and MB were effective to improve N2 fixation significantly. In uninoculated control plant, only 52% of N in the plant derived from N2 fixation while those from MA and MB inoculated plants were more than 93%.

Though MA and MB were significantly different from the ability to improve shoot dry weight and shoot N uptake at R3.5 and the amount of total fixed N in DT84 soybean but these two Bradyrhizobial isolates did not differ significantly for the effect on seed yield. DT84 soybean seed yield increased about 55 and 45% over that of uninoculated control by significant inoculation of MA and MB respectively. Single inoculation of EA resulted in only 19% improvement and the difference of seed yield between EA and the control was not significant.

In Hinthada soybean, though the tested treatments affect significantly nodule dry weight, root dry weight, shoot dry weight at both V6 and R3.5 stage, measured N2 fixing parameter at R3.5 stage (RUI) and calculated percentage of fixed N (%P) (data not shown) but in this Myanmar soybean the significant correlations between RUI and the following parameters, nodule dry weight and shoot N uptake at R3.5 stage and seed yield were found. Furthermore, correlation between root dry weight and shoot N uptake of this soybean variety at R3.5 was also significantly (Table 4).

In SJ5 soybean, the studied treatments did not have significant effects on nodulation and N2 fixing parameters but RUI correlated significantly with yield. Significant correlation between root nodule dry weight and shoot N uptake at R3.5 stage was also observed (Table 5).

Table 4. Correlation coefficient between RUI and nodule dry weight (NDW), root dry weight (RDW), shoot dry weight (SDW), shoot N (SN) at R3.5 and seed yield of DT84, SJ5 and Hinthada soybean varieties.

Parameters	DT84	SJ5	Hinthada
NDW	0.51*	-0.20	0.57*
SDW	0.54*	0.13	0.27
SN	0.65**	0.24	0.48*
Seed Yield	0.74**	0.56*	0.49*
RDW	0.51*	0.34	0.18

* = significant at $P< 0.05$; ** = significant at $P < 0.01$; n = 18

Table 5. Correlation coefficient between shoot N uptake, root dry weight (RDW) and nodule dry weight (NDW) at R3.5 stage for DT84, SJ5 and Hinthada soybean variety.

Parameters	DT84	SJ5	Hinthada
NDW	0.56*	0.18	0.38
RDW	0.41	0.57*	0.51*

* = significant at P< 0.05
** = significant at P < 0.01
n = 18

In DT84 soybean variety, significant correlation between RUI and the following parameters, nodule, root and shoot dry weight and shoot N uptake at R3.5 stage and seed yield (Table 3). Nodule dry weight at R3.5 stage was correlated significantly also with shoot N uptake (Table 4).

DISCUSSION

In all pot experiments conducted, soybean plants were grown in one hour-autoclaved soil in open field using ordinary tap water for irrigation through out experimental period but the plants from non bradyrhizobial inoculated treatments (control and EA) could form root nodules indicating incompletely soil sterilization and contamination of root nodule bacteria from open field. Theies et al., (1991 a,b) reported that legumes response to inoculation was largely dependent on the number of *Rhizobia* already established in the soil, the availability of soil N, and the demand for N by the crop (r=0.92). Fifty-nine percent of the observed variation in inoculation response trails could be explained by the numbers of soil *Rhizobia*. A decline in inoculation response was observed for soybean when the number of *Rhizobia* was >10 g^{-1}soil (Weaver and Frederick, 1974).

In this study, the population of Bradyrhizobia in the soil used might be over 20 g-1, thus significant effects of bradyrhizobial inoculation on nodule dry weight were not found. However, the inoculated bradyrhizobial isolate might be more effective than the natural Bradyrhizobia resulted in significant effects of Bradyrhizobial isolates on some studied parameters depended on the tested soybean varieties. Wani et al., (1995) reported that within grain legumes species genotypic variability affecting traits like nodule number and mass and nitrogenase activity has been observed. Significant variation in root nodule bacterial strains effectiveness has been reported by several authors (Hobbs and Mahon, 1982; Bremer et al., 1990; Rosas et al., 1998). In this study, the difference soybean varieties had different responsive pattern to Bradyrhizobial isolates which supports the report of Wani et al., (1995). The experimental result on the significant difference of the effectiveness between MA and MB Bradyrhizobial isolates on N uptake of shoot and amount of seasonal fixed N of the three test soybean varieties confirmed the studies of Hobbs and Mahon, 1982; Bremer et al., 1990; Rosas et al., 1998. According to the Spaink (1992) bateria of the genera *Bradyrhizobium, Rhizobium,* *Azorhizobium, Allorhizobium, Mesorhizobium and Sinorhizobium* could produce nod factors which are bacteria-to-plant signal molecules during the formation of Rhizobia-legume N2 fixing symbiosis. These signal molecules are induced in response to plant-to-bacteria signal molecules, which are usually flavonoids and isoflavonoids found in legume root exudates. Under green house experiment, Souleimanov et al., (2002) found out nod factor of *B. japonicum* had effects on root growth resulted in 34-44% longer root in soybean. Significant effects on root biomass improvement in Pea and Lentail by seed treatment with strains of *Rhizobium legumanosarum* bv. *viceae* under field experiment were reported also by Huang and Erickson (2006).

In this study single inoculation of MA and MB Bradyrhizobial isolates resulted in significant increase of root dry weight of SJ5 soybean at R3.5 stage. This experimental result supported the report of Souleimanov et al., (2002) and Huang and Erickson (2006). In Hinthada and DT84 soybean varieties, a positive trend to improve root growth by these two Bradyrhizobial isolates were observed also but the effects were not significant. It might be possible that SJ5 soybean plant produced flavonoids and isoflavonoid in root exudates which induced more production of nod factor of Bradyrhizobial isolates resulting in significant increasing of root biomass.

Since within grain legume species, genotypic variability affected nodule number or mass or nitrogenase activity as observed by Wani et al., (1995) selection for specific N highly effective combination of host plant and Rhizobia was suggested (Kessel and Harley, 2000). In this study, MB was effective Bradyrhizobial isolate for improvement of N assimilation, N_2 fixation and seed yield of both Hinthada and SJ5 soybean varieties. By single inoculation of MB, Hinthada soybean plant obtained N from N2 fixation almost 100% while SJ5 soybean obtained about 85% and N2 fixation parameter correlated significantly with seed yield. None of Myanmar Bradyrhzobial isolates have been tested for their effectiveness under pot trails using soil as growth substrate. This study is the first report on effectiveness of Myanmar Bradyrhizobial isolates on N_2 fixation and seed yield. However, further testing by field experiment is still needed in order to have more reliable data on the usefulness of this *Bradyrhizobium*.

In SJ5 soybean, the selected EA had significant effect only on shoot dry weight improvement at V6 stage but on the other studied parameters such as seed yield, shoot N uptake and amount of fixed N throughout the growing season, only the positive trends to improve about 31%, 12% and 40% respectively were found. From previous study of Thapanapongworakul (2003) this selected EA had a trend to improve N uptake of the whole plant of SJ5 soybean about 83% compared to uninoculated control treatment and dual inoculation of this EA and USDA 110 bradyrhizobial strain improved N uptake (443%) as much as that of single inoculation of that bradyrhizobial strain. The effectiveness of single inoculation in EA to improve the growth and N2 fixation of the tested SJ5 soybean from this study was not clearly shown even though a similar positive trend was found. Regarding to compatibility of EA with *Bradyrhizobia*, the experimental result from EA+MA agreed with Thapanapongworakulís report but opposite result was found in EA+MB. In Hinthada soybean, the similar trends of dual inoculation of EA and Bradyrhizobial isolates as found in SJ5 soybean were observed too.

Samac et al., (2003) studied the effects of antibiotics-producing *Streptomyces* on nodulation of alfalfa and found out that eight of the 15 *Streptomyces* strains inhibited invitro growth of five or more of *S. meliloti* strains, while four *Streptomyces* strains had no effect on growth of any test strains. In a growth chamber assay, two of six *Streptomyces* strains, when inoculated into the planting mix, significantly reduce plant dry weight compared to the treatment with *S. meliloti* alone, but did not significantly reduce the numbers of nodule. The other strains with strong in vitro antibiosis activity did not affect plant weight significantly. They suggested that careful selection of *Streptomyces* isolates for use in biological control of plant diseases will limit the potential negative impacts on *Rhizobia*. Our studies supported suggestion of Samac et al., (2003).

In this study, the depressive effect of EA in dual inoculated with Bradyrhizobial isolates might not be due to growth inhibition by antibiotic production of EA because such effect varied with soybean variety. Samac et al., (2003) found out that alfalfa leaves, roots and nodules were colonized by *Streptomeces* following inoculation of the planting mixture. Relatively high population density of *Streptomeces* occurred on alfalfa leaves up to 8 weeks after planting, when leaves had begun to senesce. They proposed that alfalfa plants or the rhizosphere soil of the plants provide sufficient nutrients to support development of relatively high population density of *Streptomeces* without affecting plant biomass accumulation. In this study, depressive effect of EA in dual inoculation with Bradyrhizobial isolate on shoot dry weight or N uptake or amount of fixed N might be due to completion of nutrients provided by the host plants or in the rhizosphere soil between EA and Bradyrhizobial isolates. Such depressive effect of dual inoculation of EA and Bradyrhizobial isolate were clearly shown in DT84 soybean which is the short duration crop (75 days from sowing to harvest) supporting our hypothesis. Tokala et al., (2002) reported that plant-microbe rhizosphere interaction involving root-colonizing actinomycete (*Streptomeces lydicus* WYEC (108) and the pea plant is very important to the health and growth of this nodulating legume. *S. lydicus* WYEC 108 influences pea root nodulation by increasing root nodulation frequency. Colonization leads to an increase in the average size of the nodules that form and improves the vigor of the bacteriods within the nodules by enhancing nodular assimilation of iron and possibly other soil nutrients. In this study, a trend of EA+MA to produce synergistic effect on nodule dry weight, RUI and amount of seasonal fixed N were observed in Hinthada soybean variety.

CONCLUSION

Our study showed that the effectiveness of MA and MB Myanmar Bradyrhizobial isolates to improve growth, N_2 fixation and seed yield of soybean depended on soybean varieties. In Hinthada and SJ5 soybean varieties, MB was significant better than MA when single inoculation was used. MA was more effective than MB only in DT84 soybean for improvement shoot dry weight and

N uptake of shoot at R3.5 stage and amount of fixed N throughout the growing season but not different significantly from MB for the effect on seed yield.

Single inoculation of EA, the selected endophytic actinomycetes did not have significant effects on all studied parameters in all three tested soybean varieties. Dual inoculation with EA and MB was not effective compared with single inoculation of MB and even produced significant depressive effect on amount of total fixed N for these three soybean varieties. Significant depressive effect of dual inoculation with EA and MA was found also in DT84 soybean.

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