

CHAPTER IV

RESULTS

4.1 Isolation of root nodule bacteria from Myanmar Soil

Only soybean plants grown on sterile sand and Bagan soil could form nodules but none from the other soils. Two isolates of root nodule bacteria, MA and MB, which showed typical colony characteristics of *Bradyrhizobium* in yeast mannitol congo red agar (YMA) such as forming gummy colony without absorption of congo red dye, having domed shape with smooth margin. The cells of both selected isolates were Gram negative rods. These two root nodule bacteria isolates formed single colony after streaking on YMA for at least 5 days so they were considered as bradyrhizobial isolates.

4.2 Effectiveness testing experiment

The effect of selected endophytic actinomycetes and each of bradyrhizobial isolates or strains on dry weight of the whole plants of different soybean varieties were shown in Table 2.

It was found that USDA 110 and THA 7 could increase significantly the whole plant dry weight of Myanmar soybean about 113% and 137% over that of the control. MA and MB isolates were not effective to improve the growth of Myanmar soybean significantly but these two isolates showed a trend to increase the whole plant of Myanmar soybean about 50% and 56% over the control respectively and MB did not differ significantly from USDA 110 and THA 7. EA treatment was significantly effective to improve the whole plant dry weight of Myanmar soybean

compared to the control and this treatment did not differ significantly from three bradyrhizobial treatments, USDA 110, THA 7 and MB.

USDA 110 and THA 7 were also significantly effective to increase the whole plant dry weight of SJ 5 soybean from Thailand. With these two bradyrhizobial treatments, dry weight of the whole plant of SJ 5 soybean increased 144% and 106% over that of the control. Inoculation of MA and MB resulted in increasing of dry biomass of the whole plant of SJ 5 about 31% and 64% over the control but only MB was significantly effective. MB was not different significantly from THA 7 also.

Table 2. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on whole plant dry weight (g/plant) of Myanmar, Thailand and Cambodia soybean varieties

Treatment	Whole plant dry weight (g/plant) of Soybean varieties		
	Myanmar	Thailand	Cambodia
Control	0.2325 d (100) ⁽²⁾	0.2275 d (100) ⁽²⁾	0.1625 (100) ⁽²⁾
EA	0.4375 abc (188)	0.2550 cd (112)	0.2725 (168)
MA	0.3475 cd (150)	0.2975 cd (131)	0.2750 (169)
MB	0.3625 bcd (156)	0.3850 bc (169)	0.2075 (128)
USDA110	0.4950 ab (213)	0.5550 a (244)	0.1975 (121)
THA7	0.5500 a (237)	0.4675 ab (206)	0.2725 (168)
F-test	**	**	NS
CV (%)	23.00	24.73	47.05

⁽¹⁾ Means of 4 replications;

NS = Non-significant; ** = Significant at P<0.01

⁽²⁾Figures in parenthesis were relative yield as compared to the control

EA inoculation was not effective with SJ 5 soybean and this treatment showed a trend to improve the whole plant dry biomass of this variety only 12% over the control.

All bradyrhizobial inoculated treatments and EA did not show significant effects on growth improvement of Cambodian soybean. However all of them showed a trend to increase dry weight of the whole plant over the control as follow: EA 68%, MA 69%, MB 28%, USDA 110 21% and THA 7 68%.

4.3 Pot experiments

4.3.1 Nodulation, root and shoot dry weight

4.3.1.1 Myanmar soybean

At V6 and R3.5 stages, local recommended variety from lower Myanmar responded significantly to the tested treatments for nodule and shoot dry weight as shown in Table 3 and 4 respectively. Though no Bradyrhizobial inoculation was not applied and the autoclaved soil was used for soybean cultivation but soybean plants from the treatments as follows; control, EA, N and EA + N produced root nodule. However the weights of nodule from those treatments were comparative less than those from inoculated treatments. Single inoculation of MA, MB, USDA 110 and THA 7 were not different from each other for the effects on nodule dry weight at both growth stage but only MB and USDA 110 were significantly effective to increase nodule dry weight compare to the control. Dry weight of nodule from MB and USDA 110 inoculated plants at V6 stage were about 77% and 59% over that of the control and about 65% and 62% at R3.5 stage. MA and THA 7 showed a trend to improve dry weight of nodule about 41% and 41% at V6 and about 23% and 42% over the control

Table 3. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on nodule, root and shoot dry weight (g/plant) of Myanmar soybean variety at V 6 stage

Treatment	V6		
	Nodule dry weight	Root dry weight	Shoot dry weight
Control	0.17 e (100) ⁽²⁾	1.34 (100) ⁽²⁾	3.29 c (100) ⁽²⁾
EA	0.19 de (112)	2.18 (163)	3.59 c (109)
MA	0.24 abcde (141)	1.53 (114)	3.48 c (106)
MB	0.30 ab (177)	2.44 (182)	5.39 ab (164)
USDA110	0.27 abcd (159)	2.35 (175)	5.33 ab (162)
THA7	0.24 abcde (141)	2.28 (170)	4.49 bc (136)
N	0.21 bcde (124)	2.13 (159)	3.53 c (107)
EA+N	0.18 de (106)	2.07 (154)	4.06 bc (123)
EA+MA	0.20 cde (118)	1.95 (146)	3.96 bc (120)
EA+MB	0.24 abcde (141)	2.20 (164)	4.56 bc (139)
EA+ USDA110	0.31 a (182)	2.52 (188)	6.31 a (192)
EA+ THA7	0.30 abc (177)	2.36 (176)	5.34 ab (162)
F-test	*	NS	*
CV (%)	24.04	23.70	22.96

⁽¹⁾ Means of 3 replications

NS = Non-significant; * = Significant at P<0.05

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

at R3.5 stage. Soybean plant treated with N fertilizer also showed a trend to improve nodule dry weight about 24% over the control but such trend was found only at V6.

Table 4. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on nodule, root and shoot dry weight dry weight (g/plant) of Myanmar soybean variety at R 3.5 stage

Treatment	R 3.5		
	Nodule dry weight	Root dry weight	Shoot dry weight
Control	0.26 e (100) ⁽²⁾	2.60 (100) ⁽²⁾	6.72 e (100) ⁽²⁾
EA	0.29 cde (112)	3.32 (128)	7.88 bcde (117)
MA	0.32 bcde (123)	2.90 (112)	7.51 cde (112)
MB	0.43 ab (165)	3.68 (142)	10.28 a (153)
USDA110	0.42 ab (162)	3.48 (134)	9.41 abc (140)
THA7	0.37 abcde (142)	3.18 (122)	9.28 abcd (138)
N	0.27 de (104)	3.53 (136)	7.12 de (106)
EA+N	0.27 de (104)	3.56 (137)	6.75 e (100)
EA+MA	0.40 abc (154)	3.19 (123)	9.12 abcd (136)
EA+MB	0.38 abcd (146)	3.14 (121)	9.79 abc (146)
EA+ USDA110	0.47 a (181)	4.06 (156)	10.75 a (160)
EA+ THA7	0.45 a (173)	3.56 (137)	9.95 ab (148)
F-test	**	NS	*
CV (%)	19.53	16.26	15.51

⁽¹⁾ Means of 3 replications; ^{NS} = Non-significant;

* = Significant at P<0.05; ** = Significant at P<0.01

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

Inoculation of EA in combination with Bradyrhizobial isolate or strains or with N fertilizer did not show significant effects on nodulation compared to single inoculation treatment. However the positive trend to improve nodule dry weight by dual inoculation were observed in the following combinations, EA + USDA 110 and EA + THA 7 at both V6 and R3.5 stages, EA + MA at V6 stage while the use of EA + MA showed such trend only at V6 stage. Dual inoculation EA with MB showed a trend to reduce nodule dry weight at both V6 and R3.5 stages.

Though the significant effects of the tested treatments on root dry weight of Myanmar soybean at V6 and R3.5 stages were not found, all single Bradyrhizobial inoculated treatments including single inoculation of EA and N applied treatment showed a trend to improve root dry weight at both growth stages compared to that of the control. The positive trend to improve root biomass by dual inoculation was found also in the following combinations, EA + USDA 110 and EA + THA 7 at V6 stage. The use of EA in combination with Myanmar Bradyrhizobial isolates showed a trend to reduce root biomass at V6 stage compared to those from single inoculation but at R3.5 stage only EA + MA treatment showed a trend to improve root biomass slightly. The use of EA in combination with N fertilizer did not affect root biomass compared with those from EA or N applied treatment.

Regarding to shoot dry weight, it was found that single inoculation of MB and USDA 110 could improve significantly shoot dry weight of Myanmar soybean at V6 stage about 64% and 62% respectively compared to that of the control while single inoculation of EA, MA and THA 7 showed only a trend to have positive effect on shoot biomass. Myanmar soybean did not show significant respond to N application at both V6 and R3.5 stage and shoot biomass increased less

than 10% by this treatment. The effects of dual inoculation of EA and each of bradyrhizobial isolate or strain on shoot dry weight at both stages were not significant but the positive trend to improve shoot biomass were observed in some combination such as EA + USDA 110 and EA + MA at both V6 and R3.5 stage compared to single inoculation treatment. The use of EA together with N fertilizer did not show any beneficial effect on shoot biomass of Myanmar soybean at V6 and R3.5 stages.

4.3.1.2 Thailand soybean

Data on nodule, root and shoot dry weight of SJ 5 soybean variety from Thailand at V6 and R3.5 stages were shown in Table 5 and 6. Nodule formations by Thailand soybean variety in non bradyrhizobial inoculated treatments were observed. Single inoculation of MB, USDA 110 and THA 7 were not significantly different from each other for the effects on nodule dry weight and these three treatments were all effective to improve nodule dry weight of SJ 5 soybean at V6 stage about 86%, 59% and 64% compared to that of the control ($P < 0.05$). The following treatments: EA, MA and N showed only a trend to increase nodule dry weight about 9% but each treatment was not different significantly from the control and single inoculation of USDA 110 and THA 7.

The use of EA in combination with N fertilizer or bradyrhizobial inoculation did not show any beneficial effect on nodule dry weight compared to single inoculation except one case EA + THA 7 which showed a trend to improve nodule formation. In SJ 5 soybean, no significant effects of the tested treatments on root dry weight at both V6 and R3.5 were found but single inoculation of MB, USDA 110 and THA 7 showed the trend to increase root biomass at V6 about

47%, 46% and 42%, and about 72%, 63% and 80% at R3.5 stage. Single inoculation of MA showed also the positive trend to improve root biomass particularly at R3.5 about 61% compared to that of the control. In SJ 5 soybean, EA inoculation without *Bradyrhizobium* was not effective for root formation at both V6 and R3.5 stage but when EA was used together with some Bradyrhizobia such as USDA 110 and THA 7 a trend of synergistic effect of dual inoculation on root biomass improvement were observed at R3.5 stage.

Not only root biomass at R3.5 stage synergistic effect of dual inoculation of EA and USDA 110 and THA 7 on nodule dry weight were also observed particular EA + USDA 110 which the effect on root nodule dry weight improvement was significant compare to that single inoculation and the control.

Among single inoculated treatments, only MB and THA 7 were significantly effective to compare shoot dry weight of SJ 5 soybean at V6 stage but the rest showed only a trend to improve shoot biomass.

Nevertheless, there were no significant differences of shoot dry weight at V6 stage among single inoculated treatments. In SJ 5 soybean, single inoculation of EA was not effective to improve significantly shoot biomass but EA showed a trend to increase shoot biomass of SJ 5 soybean about 33% over that of the control at V6 stage but such trend was not found at R3.5. Surprisingly, N- applied treatment was not effective for shoot biomass improvement of SJ 5 both at V6 and R3.5 stage both with and without EA inoculation. Though dual inoculated treatments were not different significantly from single inoculated ones but two treatments, EA + USDA 110 and EA + THA 7 showed a trend to have synergistic effect on increasing of shoot biomass of SJ 5 soybean at both V6 and R3.5 growth stages.

Table 5. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on nodule, root and shoot dry weight dry weight (g/plant) of Thailand soybean variety at V 6 stage

Treatment	V6		
	Nodule dry weight	Root dry weight	Shoot dry weight
Control	0.22 d (100) ⁽²⁾	1.69 (100) ⁽²⁾	3.76 e (100) ⁽²⁾
EA	0.24 cd (109)	1.80 (107)	5.01 cde (133)
MA	0.24 cd (109)	1.81 (107)	5.23 bcde (139)
MB	0.41 ab (186)	2.48 (147)	6.64 ab (177)
USDA110	0.35 abc (159)	2.46 (146)	5.24 bcde (139)
THA7	0.36 abc (164)	2.38 (141)	6.46 abc (172)
N	0.24 cd (109)	1.83 (108)	3.88 de (103)
EA+N	0.24 cd (109)	2.18 (129)	4.16 de (111)
EA+MA	0.25 cd (114)	2.30 (136)	4.12 de (110)
EA+MB	0.28 bcd(127)	2.18 (129)	5.27 bcd (140)
EA+ USDA110	0.35 abc(159)	2.21 (131)	5.79 abc (154)
EA+ THA7	0.42 a (191)	2.66 (157)	7.10 a (189)
F- test	*	NS	**
CV (%)	24.90	20.72	16.77

⁽¹⁾ Means of 3 replications; ^{NS} = Non-significant;

* = Significant at P<0.05; ** = Significant at P<0.01

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

Table 6. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on nodule, root and shoot dry weight (g/plant) of Thailand soybean variety at R 3.5 stage

Treatment	R 3.5		
	Nodule dry weight	Root dry weight	Shoot dry weight
Control	0.33 b (100) ⁽²⁾	2.39 (100) ⁽²⁾	7.08 c (100) ⁽²⁾
EA	0.34 b (103)	2.53 (106)	7.26 c (103)
MA	0.34 b (103)	3.84 (161)	8.29 bc (117)
MB	0.39 b (118)	4.10 (172)	12.19 a (172)
USDA110	0.37 b (112)	3.89 (163)	11.38 ab (161)
THA7	0.53 ab (161)	4.31 (180)	12.21 a (172)
N	0.34 b (103)	3.81 (159)	7.13 c (101)
EA+N	0.34 b (103)	2.93 (123)	7.36 c (104)
EA+MA	0.35 b (106)	2.91 (122)	9.61 abc(136)
EA+MB	0.34 b (103)	3.82 (160)	10.46abc (148)
EA+ USDA110	0.62 a (188)	4.51 (189)	12.73 a (180)
EA+ THA7	0.63 a (191)	4.57 (191)	13.27 a (187)
F- test	*	NS	*
CV (%)	31.29	20.85	22.79

⁽¹⁾ Means of 3 replications;

NS = Non-significant; * = Significant at P<0.05

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

4.3.1.3 Cambodian soybean

The data on nodule, root and shoot dry weight of Cambodia soybean at V6 and R3.5 stages were shown in Table 7-8. The plants from non bradyrhizobial inoculated treatments of Cambodia variety could form nodules as well as the other two soybean varieties mentioned previously.

All bradyrhizobial inoculated treatments were not different significantly from each other for the effects on nodule dry weight of Cambodian soybean at V6 and R 3.5 stages but only 3 bradyrhizobial treatments could increase significantly nodule dry weight over the control. They were MB, USDA 110 and THA 7 for V6 stage and MA, USDA 110 and THA 7 for R 3.5 stage. At V6 stage, nodule dry weight of Cambodian soybean increased within the range of 72%-88% by those three effective bradyrhizobial treatments and at R3.5 were about 52%-60% over those from the control treatment. Cambodian soybean did not respond significantly to EA inoculation and N- application for nodule, root and shoot dry weight.

Dual inoculation of EA and each of *Bradyrhizobium* isolate/ strain did not support beneficial effect but depress nodule formation and development of shoot and root. Among the tested bradyrhizobial isolates/ strains, MB seemed to be more sensitive to EA than the others. Nodule dry weight from EA + MB inoculated Cambodian soybean was significantly lower than that from single MB inoculated plant while the other combination treatments showed a trend to reduce.

No significant effect of single inoculation of EA or each of bradyrhizobial inoculated treatment on root dry weight were found at both V6 and R3.5 stage. However, single inoculation of bradyrhizobial isolate/strain showed a trend to improve root dry weight of Cambodian soybean within the range of 8%-29%

Table 7. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on nodule, root and shoot dry weight dry weight (g/plant) of Cambodian soybean variety at V 6 stage

Treatment	V6		
	Nodule dry weight	Root dry weight	Shoot dry weight
Control	0.25 c (100) ⁽²⁾	1.64 (100) ⁽²⁾	5.01 b (100) ⁽²⁾
EA	0.26 c (104)	1.76 (107)	5.25 b (105)
MA	0.32 abc (128)	1.77 (108)	5.50 b (110)
MB	0.46 a (184)	1.92 (117)	7.84 a (157)
USDA110	0.47 a (188)	2.12 (129)	8.14 a (162)
THA7	0.43 ab (172)	2.06 (126)	7.78 a (155)
N	0.27 c (108)	1.80 (110)	5.40 b (108)
EA+N	0.29 bc (116)	1.81 (110)	5.50 b (110)
EA+MA	0.28 bc (112)	1.87 (115)	5.80 b (116)
EA+MB	0.27 c (108)	1.88 (115)	5.54 b (111)
EA+ USDA110	0.37 abc(148)	1.96 (120)	5.89 b (118)
EA+ THA7	0.34 abc(136)	2.24 (137)	5.87 b (117)
F- test	*	NS	**
CV (%)	27.99	14.03	12.38

⁽¹⁾ Means of 3 replications; ^{NS} = Non-significant;

* = Significant at P<0.05; ** = Significant at P<0.01

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

Table 8. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on nodule, root and shoot dry weight dry weight (g/plant) of Cambodian soybean variety at R 3.5 stage

Treatment	R 3.5		
	Nodule dry weight	Root dry weight	Shoot dry weight
Control	0.25 d (100) ⁽²⁾	2.49 (100) ⁽²⁾	7.35 d (100) ⁽²⁾
EA	0.25 d (100)	2.69 (108)	8.51 cd (116)
MA	0.40 a (160)	3.15 (127)	12.39 a (168)
MB	0.37 abcd (148)	2.92 (117)	9.51 bcd (129)
USDA110	0.38 abc (152)	2.94 (118)	10.58 abc (144)
THA7	0.40 ab (160)	3.34 (134)	11.36 ab (154)
N	0.25 d (100)	2.61 (105)	8.11 cd (110)
EA+N	0.26 cd (104)	2.60 (104)	8.49 cd (116)
EA+MA	0.27 cd (108)	3.05 (122)	9.47 bcd (129)
EA+MB	0.26 d (104)	2.79 (112)	8.62 cd (117)
EA+ USDA110	0.28 bcd (112)	2.83 (114)	9.55 bcd (130)
EA+ THA7	0.28 bcd (112)	3.18 (128)	10.56 abc (144)
F- test	*	NS	*
CV (%)	23.11	17.93	16.34

⁽¹⁾ Means of 3 replications;

NS = Non-significant; * = Significant at P<0.05;

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

over that of the control while EA showed a trend to improve about 7%. Synergistic trend of dual inoculation of EA and Bradyrhizobia were not observed except one case EA + THA 7 only slightly at V6 stage.

Significant beneficial effects of single inoculation of MB, USDA 110 and THA 7 on shoot biomass improvement of Cambodian soybean were observed at V6 stage compared to the control but non for EA, N and dual inoculation treatments except EA + THA 7. There were no significant different among single inoculation of MB, USDA 110 and THA 7 and these three treatments could improve shoot biomass of Cambodian soybean at V6 stage about 55%-62% over that of the control. When dual inoculated treatments were used, shoot dry weight of the plant using dual inoculation of EA and each of the following Bradyrhizobia: MB, USDA 110 and THA 7 reduced significantly compared with those from single inoculated treatments. At R3.5 stage, MA which did not effectively function during the vegetative growth was more active while MB's activity seemed to be depressed. Thus, the effective single bradyrhizobial inoculated treatments being able to improve significantly shoot biomass of Cambodian Soybean were MA, USDA 110 and THA 7 with biomass over the control about 68%, 44% and 54%. At this stage, single inoculation of EA or in combination with N fertilizer did not effectively improve Cambodian soybean shoot biomass. The use of EA together with each of bradyrhizobial inoculation had a trend to reduce shoot biomass at R3.5 stage. Such depressive effect of dual inoculation on shoot biomass of Cambodian soybean at this growth stage was significant in EA + MA treatment compared to that of single inoculated one.

4.3.2 N₂ fixation and N uptake of soybean at R3.5 stage

Relative ureide indices (RUI) were used to indicate activity of N₂ fixation of Bradyrhizobia in root nodule and the percentage of N derived from N₂ fixation within the growing season of soybean each treatment was calculated from RUI values at R3.5 stage as suggested by Herridge and People (2002).

4.3.2.1 Myanmar soybean

The data on relative ureide indices, percentage of N derived from N₂ fixation % N fixed, total N accumulation of the above ground part and amount of fixed N of Myanmar soybean were shown in Table 9.

At R3.5 stage there were no significant different of RUI of root bleeding sap of Myanmar soybean treatments without bradyrhizobial inoculation as follow: control, EA, N, EA + N. The RUI values of those treatments were within the ranges of 56%-61%. By such RUI values derived 61%-67% of N in the plant was derived from N₂ fixation. Single inoculation of EA though did not improve N₂ fixation of soybean but this treatment had a trend to increase N₂ fixation about 10% over that of the control. Among single bradyrhizobial inoculated treatments, the best treatment was MB which gives RUI about 88%. However, MB did not differ from USDA 110 and THA 7 significantly for both RUI and %P-fix. MA was the poorest but not differed significantly from USDA 110 and THA 7. The percentage of fixed N through out the growing season from MA, MB, USDA 110 and THA 7 inoculated soybean plants were about 81%, 100%, 94% and 84% of the total N in the plant, respectively. At R3.5 stage, the second half portion of N fertilizer was not applied yet. Thus the RUI and % P-fix of soybean plants from N and EA + N treatments were

almost similar to that of the control plant. Dual inoculation of EA and each of the following

Table 9. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on relative ureide indices at R3.5 stage (%), percentage and amount of seasonal fixed N P-fix (%) and Total N accumulation in the shoot and amount of fixed N of Myanmar soybean variety

Treatment	Relative ureide index (%)	Seasonal fixed N P-fix (%)	Shoot N	
			accumulation amount (mg N/plant)	Amount of fixed N (mg N/plant)
Control	55.81 f (100) ⁽²⁾	61.46 f (100) ⁽²⁾	152 c (100) ⁽²⁾	92.33 d (100) ⁽²⁾
EA	60.77 def(109)	67.43 def(110)	177 c(116)	119.33 d (129)
MA	71.84 cde(129)	80.77 cde(131)	180 c(118)	145.39 cd (157)
MB	87.74 ab(157)	99.93 ab(163)	513 ab(338)	513.00 ab (556)
USDA110	83.11 abc(149)	94.35 abc(154)	287 bc(189)	270.78 bcd(293)
THA7	74.23 bcd(133)	83.66 bcd(136)	283 bc(186)	236.76 bcd(256)
N	57.90 ef(104)	63.98 ef(104)	153 c(101)	97.00 cd (105)
EA+N	57.47 ef(103)	63.45 ef(103)	153 c(101)	99.33 cd (108)
EA+MA	81.08 abc(145)	91.90 abcde(150)	227 c(149)	208.61 cd (226)
EA+MB	76.37 bc(137)	86.23 bc(140)	340 bc(224)	294.00 bcd(318)
EA+USDA110	93.64 a(168)	100 a(163)	667 a(439)	667.00 a (722)
EA+ THA7	90.03 a(161)	100 ab(163)	337 bc(222)	337.00 bc (365)
F- test	**	**	**	**

CV (%)	12.26	13.10	47.34	52.17
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⁽¹⁾ Means of 3 replications; ** = Significant at P<0.01

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

Bradyrhizobium: MA, USDA 110 and THA 7 resulted in higher RUI values compared to the single inoculation of each *Bradyrhizobium* but the significant differ synergistic effects of dual inoculation was found in EA + THA 7 only. When EA was used together with MA, MB, USDA 110 and THA 7, the percentage of seasonal fixed N were about 92%, 86%, 100% and 100% of the total N in the whole plant respectively. At R3.5 stage, there were no significant differences on total N accumulated in the shoot of Myanmar soybean among the following treatments: control, EA, N and EA + N. The total N uptake of these treatments was within the ranges of 153-177 mg N/plant. Single inoculation of *Bradyrhizobium* isolates or strains resulted in increasing of total N uptake of shoot about 18%-338% over that of the control but only MB inoculated treatment produced significant stimulating effect. Dual inoculation of EA and the following *Bradyrhizobium*: MA, USDA 110 and THA 7 had synergistic effect on shoot N uptake compare to single inoculated treatment but only EA + USDA 110 produced significant synergistic effect. The use of EA and MB showed a trend to have depressive effect on Myanmar soybean shoot N uptake.

Regarding to the amount of fix N, it was found that in the control Myanmar soybean plant, about 92 mg N came from N₂ fixation. Single inoculation of EA had a trend to increase amount of fixed N about 29% of that from the control but the difference was not significant. No significant effect of N- fertilizer both with or without EA inoculation on the amount of fixed N of Myanmar soybean were found but slight increase about 5%-8% over the control by these two treatments

were observed. Among single inoculation of *Bradyrhizobium*, MB was the best for increasing of the amount of fixed N about 5 times of the control. Anyhow, this treatment was not different significantly from USDA 110 and THA 7 which improve amount of fixed N about 2.9 and 2.6 times of the fixed N in the control treatment. Dual inoculation of EA and each of *Bradyrhizobium*, MA, USDA 110 and THA 7 showed synergistic effect on improvement the amount of fixed N compared to single inoculation but only EA + USDA 110 had significant effect.

4.3.2.2 Thailand soybean

RUI, % P-fix, N accumulation of shoot and amount of fixed N of SJ 5 soybean variety from Thailand were shown in Table 10.

SJ 5 soybean from the control treatment had RUI value of root bleeding sap at R3.5 stage about 50%. No effect of single inoculation of EA, N, EA + N treatments on RUI as found in Myanmar soybean were found also in SJ 5. There were no significant differences among single inoculation of bradyrhizobial treatments but MB, USDA 110 and THA 7 were significantly improved RUI value of SJ 5 at R3.5 stage. The percentage of seasonal fixed N of SJ 5 soybean inoculated with bradyrhizobial isolates or strains were within the ranges of 75%-87% and significant differences among them were not found. Nevertheless, the percentage of fixed N from MB, USDA 110 and THA 7 were significantly higher than that of the control. The effects of dual inoculation of EA and each of *Bradyrhizobium* on percentage of fixed N was not different significantly from single inoculation of *Bradyrhizobium*. However, EA + USDA 110 and EA + THA 7 showed a trend to have synergistic effect on improvement of %P-fix.

No significant effect of EA, N and EA + N treatments on N uptake of SJ 5 soybean were found but markedly improvement were observed in

Table 10. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on relative ureide indices at R3.5 stage (%), percentage and amount of seasonal fixed N P-fix (%) and Total N accumulation in the shoot and amount of fixed N of Thailand soybean variety

Treatment	Relative ureide index (%)	Seasonal fixed N P-fix (%)	Shoot N	
			accumulation amount (mg N/ plant)	Amount of fixed N (mg N/plant)
Control	50.33 c (100) ⁽²⁾	54.86 d (100) ⁽²⁾	107e (100) ⁽²⁾	63 d (100) ⁽²⁾
EA	57.00 bc (113)	62.89 bcd(115)	120 e(112)	72 d(114)
MA	67.00 abc(133)	74.94 abcd(137)	150 de(140)	112 cd(177)
MB	75.00 ab(149)	84.58 abc(154)	280 cd(262)	235 bc(373)
USDA110	74.33 ab(148)	83.77 abc(153)	230 cde(216)	194 bcd(308)
THA7	75.67 ab(150)	87.31 ab(159)	323 bc(302)	277 b(440)
N	54.00 bc(107)	59.28 cd(108)	110 e(103)	64 d(102)
EA+N	52.33 c(104)	57.27 d(104)	117 e(109)	64 d(102)
EA+MA	67.67 abc(134)	75.74 abcd(138)	167 de(156)	119 cd(189)
EA+MB	62.00 abc(123)	68.92 abcd(126)	200 cde(187)	137 cd(217)
EA+USDA110	82.33 a(164)	93.41 a(170)	453 ab(423)	423 a(671)
EA+ THA7	83.67 a(166)	95.02 a(173)	490 a(458)	463 a(735)

F- test	*	*	**	**
CV (%)	19.30	20.82	35.91	43.20

⁽¹⁾ Means of 3 replications; * = Significant at P<0.05; ** = Significant at P<0.01

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

MB, USDA 110 and THA 7 single inoculated treatment. These three bradyrhizobial treatments improved total shoot N uptake about 2.1-3.0 times of that in the control. Dual inoculations of EA and USDA 110 or THA 7 were even significantly better than single inoculation for improvement of shoot N uptake of SJ 5 soybean. In MB, USDA 110 and THA 7 inoculated plant, the amount of fixed N were 235, 194 and 277 mg N/ plant respectively which were about 85%, 84% and 87% of the total N uptake of shoot. The amounts of fixed N from these three Bradyrhizobial treatments were about 3-4 times of that in the control treatment. When dual inoculations of EA and USDA 110 or THA 7 were used, SJ 5 gained significantly more amount of fixed N compared to those in single inoculated treatments.

4.3.2.3 Cambodian soybean

RUI, % P- fix, N accumulation of shoot and amount of fixed N of Cambodian soybean variety from Thailand were shown in Table 11.

At R3.5 stage there was no effect of single inoculation of EA, N, EA + N treatments on RUI was similar to those of Myanmar and Thailand soybean. There were no significant differences among single inoculation of bradyrhizobial treatments but MA, MB, USDA 110 and THA 7 were significantly improved RUI value of Cambodian soybean at R3.5 stage. Single inoculation of EA though did not improve N₂ fixation of soybean but this treatment had a trend to

increase N₂ fixation about 4% over that of the control. Among single Bradyrhizobial inoculated treatments, the best treatment was MA which gives RUI about 90%. However, MA did not differ from MB, USDA 110 and THA 7 significantly for both RUI and % P-fix. The percentage of fixed N through out the growing season from

Table 11. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on relative ureide indices at R3.5 stage (%), percentage and amount of seasonal fixed N P-fix (%) and Total N accumulation in the shoot and amount of fixed N of Cambodian soybean variety

Treatment	Relative ureide index (%)	Seasonal fixed N P-fix (%)	Shoot N	
			accumulation amount (mg N/plant)	Amount of fixed N (mg N/plant)
Control	48.33c (100) ⁽²⁾	52.45c (100) ⁽²⁾	113 f(100) ⁽²⁾	65 f(100) ⁽²⁾
EA	50.33c(104)	54.86 c(105)	140 ef(124)	81 f(125)
MA	89.67 a(186)	100 a(191)	437 a(387)	437 a(672)
MB	81.67 ab(169)	92.61 ab(177)	240 bcdef(212)	222 cd(342)
USDA110	85.33 ab(177)	97.03 ab(185)	310 abc(274)	298 bc(459)
THA7	84.67 ab(175)	98.15 ab(187)	367 ab(324)	360 ab(554)
N	50.00 c(103)	54.46 c(104)	133 f(118)	72 ef(111)
EA+N	53.00 c(110)	58.37 c(111)	150 def(133)	85 ef(131)
EA+MA	70.00 abc(145)	78.56 abc(150)	180 cdef(159)	141 def(217)
EA+MB	66.33 bc(137)	74.14 bc(141)	187 cdef(165)	139 ef(214)
EA+USDA110	78.67 ab(163)	88.99 ab(170)	320 bcde(283)	285 bc(438)

EA+ THA7	67.33 bc(139)	75.34 bc(144)	250 bcd(221)	188 cde(289)
F- test	**	**	**	**
CV (%)	18.62	19.98	36.35	33.00

⁽¹⁾ Means of 3 replications; ** = Significant at P<0.01

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

MA, MB, USDA 110 and THA 7 inoculated soybean plants were about 100%, 93%, 97% and 98% of the total N in the plant respectively. There were no significant differences among dual inoculation of EA and each of the following *Bradyrhizobium*: MA, MB, USDA 110 and THA 7. The use of EA and all *Bradyrhizobium* strains showed a trend to have depressive effect on Cambodian soybean in % P-fix.

No significant effect of EA, N and EA + N treatments on N uptake of Cambodian soybean were found but markedly improvement were observed in MA, MB, USDA 110 and THA 7 single inoculated treatment. These treatments improved total shoot N uptake about 2.1-3.8 times of that in the control but only single inoculation of *Bradyrhizobium*, MA had significant effect. The total N uptake of the treatments of dual inoculations of EA and all *Bradyrhizobium* strains were not significantly different each other while EA+ USDA110 was better than the others in Cambodian soybean. In MA, USDA 110 and THA 7 inoculated plant, the amount of fixed N were 437, 298 and 360 mg N/ plant respectively. These treatments were about 100%, 97% and 98% of the total N uptake of shoot. The amounts of fixed N from these three bradyrhizobial treatments were about 4-6 times of that in the control treatment. When dual inoculations of EA and the following *Bradyrhizobium* strains; MA, MB, USDA 110 and THA 7 were used, Cambodian soybean gained a trend to

have depressive effect significantly more amount of fixed N compared to those in single inoculated treatments.

4.3.3 Seed yield and yield component of soybean

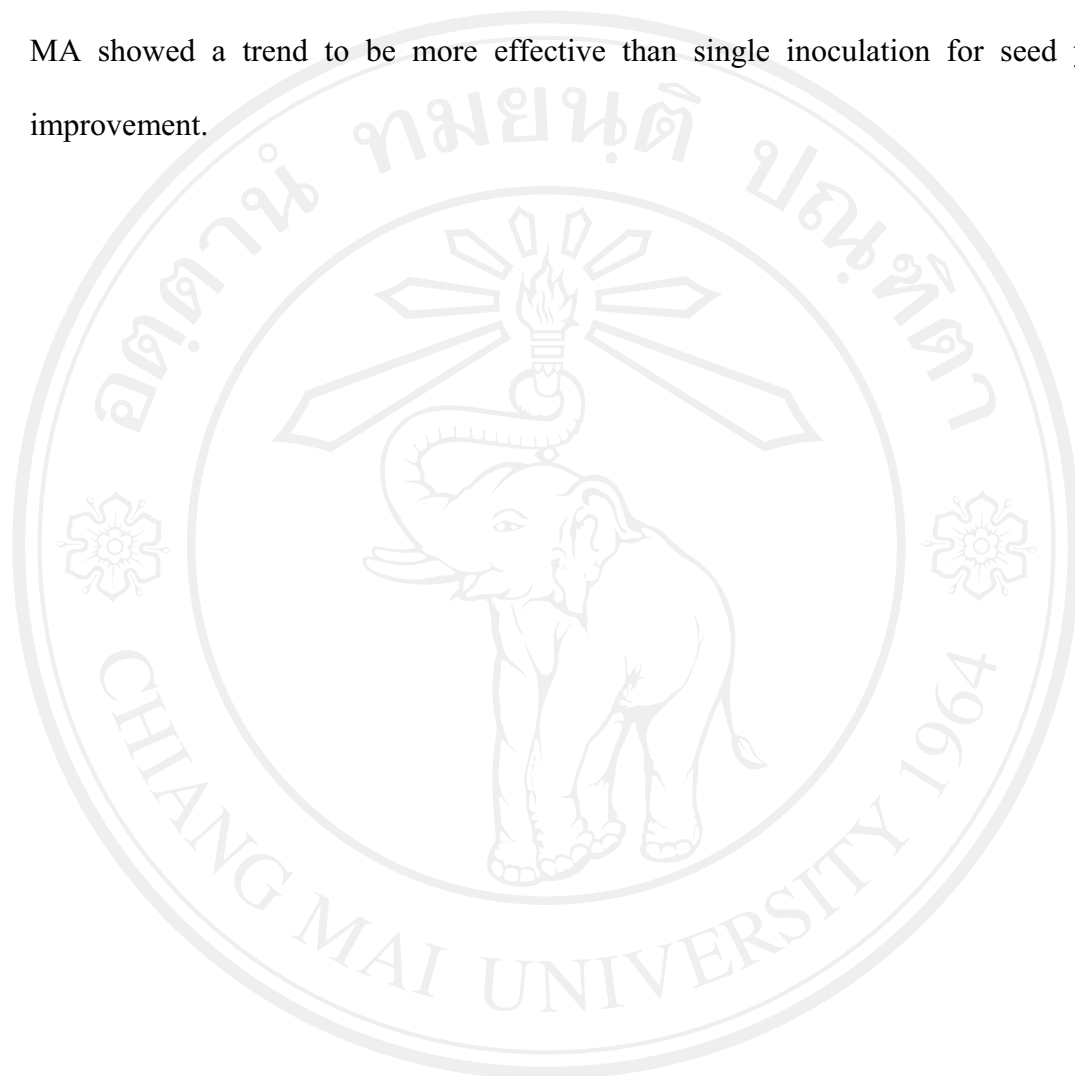
Seed yield and yield component of Myanmar, Thailand and Cambodian soybean varieties were shown in Tables 12, 13, and 14 respectively.

4.3.3.1 Myanmar soybean

Myanmar soybean (Table12) from the control treatment had about 11 pods per plant with 1.4 seed per pod and gave 9.84 g seed yield/ plant. Single inoculation of EA improved significantly (27%) the number of pod per plant compared to that of the control but had only a trend to increase number of seed per pod and seed yield about 17%. N application at rate of 6 kg N/ rai either alone or in combination with EA did not significant effects on both seed yield and studied yield component compared to the control treatments. All single inoculation of bradyrhizobial treatment except MA was effective to improve significantly. The number of pods/ plant and number of seeds/ pod resulting in seed yield increment about 96%, 77% and 79% over that of the control ($P < 0.01$). Significant differences of seed yield among these three effective bradyrhizobial treatments were not found. Dual inoculation of USDA 110 or THA 7 and EA were better than single inoculation particularly USDA 110 which synergistic effect of dual inoculation of EA and this bradyrhizobial strain on seed yield was significant.

Seed yield improvement of Myanmar soybean by dual inoculation of EA + USDA 110 and EA+ THA 7 were about 125% and 93% over the control. Though the differences of seed yield between dual and single inoculation of each

Myanmar bradyrhizobial isolate were not significant but there was a trend that MA seemed to be more compatible with EA than MB because dual inoculation of EA and MA showed a trend to be more effective than single inoculation for seed yield improvement.



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Table 12. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on pods per plant, seeds per pod and seed yield of Myanmar soybean variety at harvest stage

Treatment	Pod per plant	Seed per pod	Seed yield (g/plant)
Control	10.78f (100) ⁽²⁾	1.41 e (100) ⁽²⁾	9.84 e (100) ⁽²⁾
EA	13.67 de (127)	1.75 de (124)	11.49 e (117)
MA	13.22 def (123)	1.77 cde (125)	12.52 de (127)
MB	19.76 ab (183)	2.32 ab (165)	19.33 ab (196)
USDA110	17.33 bc (161)	2.06 abcd(146)	17.46 bc (177)
THA7	17.33 bc (161)	2.02 bcd (143)	17.61 abc (179)
N	11.33 ef (105)	1.72 de (122)	11.02 e (112)
EA+N	12.56 def (117)	1.73 de (123)	10.10 e (103)
EA+MA	15.00 c (139)	1.99 bcd (141)	14.07 cde (143)
EA+MB	16.55 cd (154)	2.03 bcd (144)	17.08 bcd (174)
EA+ USDA110	20.27a (188)	2.47 a (175)	22.09 a (225)
EA+ THA7	19.64 ab (182)	2.17 abc (154)	18.94 ab (193)
F- test	**	*	**
CV (%)	9.94	12.67	17.84

⁽¹⁾ Means of 3 replications;

* = Significant at P<0.05; ** = Significant at P<0.01

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

Table 13. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on pods per plant, seeds per pod and seed yield of Thailand soybean variety at harvest stage

Treatment	Pod per plant	Seed per pod	Seed yield (g/plant)
Control	11.78 e (100) ⁽²⁾	1.62 b (100) ⁽²⁾	8.69 e (100) ⁽²⁾
EA	13.00 bcde (110)	1.70 b (105)	11.38 cde (131)
MA	11.78 e (100)	1.72 b (106)	9.14 e (105)
MB	18.11 ab (154)	2.16 ab (133)	15.74 bc (181)
USDA110	17.00 abcd (144)	2.10 ab (130)	14.50 bcd (167)
THA7	15.22 bcde (129)	2.19 ab (135)	15.65 abc (180)
N	12.89 cde (109)	1.76 b (109)	10.54 de (121)
EA+N	12.11 de (103)	1.72 b (107)	9.83 e (113)
EA+MA	15.11 bcde (128)	2.00 ab (124)	12.55 cde (144)
EA+MB	17.33 abc (147)	2.08 ab (128)	12.83 cde (148)
EA+ USDA110	21.56 a (183)	2.40 a (148)	18.41 ab (212)
EA+ THA7	21.66 a (184)	2.55 a (157)	19.92 a (229)
F- test	**	*	**
CV (%)	19.55	17.46	19.13

⁽¹⁾ Means of 3 replications;

* = Significant at P<0.05; ** = Significant at P<0.01

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

4.3.3.2 SJ 5 soybean variety from Thailand

In control treatment, SJ 5 soybean variety had about 12 pods per plant with 1.62 seeds per pod and gave seed yield about 8.69 g/ plant. Single inoculation of EA though did not have significant effect on seed yield but showed a trend to improve seed yield about 31% over that of the control. N- application treatment either alone or in combination with EA showed only a trend to improve seed yield about 31% and 13% compared to the control respectively. Seed yield improvement of SJ 5 soybean by EA + USDA 110 and EA + THA 7 was about 112% and 129 % over that of the control.

4.3.3.3 Cambodian soybean

In the control treatment, Cambodian soybean gave seed yield about 7.07 g/ plant. Single inoculation of EA did not have significant effect on seed yield of Cambodian soybean but showed a trend to improve about 19%. There were no significant differences of seed yield among single inoculation of bradyrhizobial treatments and all could produce significantly more seed yield than the control (45%-55%). The numbers of seeds per pod were significantly increased by single inoculation of bradyrhizobial treatments. N- applied treatment was not had significant effects on seed yield and number of seed per pod but the use of N plus EA inoculation improved significantly seed yield of Cambodian soybean (32%). None of dual inoculated treatments was significantly better than single inoculation for the effect on seed yield. The number of pods per plant and the number of seeds per pod were not also affected significantly by EA, N and EA + N treatments.

There were no significant differences of seed yield among the following Bradyrhizobial treatments: MB, USDA 110 and THA 7 and all of them

Table 14. Effects⁽¹⁾ of endophytic actinomycetes and root nodule bacterial inoculation on pods per plant, seeds per pod and seed yield of Cambodian soybean variety at harvest stage

Treatment	Pod per plant		Seed per pod		Seed yield (g/plant)
Control	9.22	(100) ⁽²⁾	1.48 e	(100) ⁽²⁾	7.07 d (100) ⁽²⁾
EA	10.00	(108)	1.60 de	(108)	8.41 bcd (119)
MA	13.67	(148)	2.42a	(164)	10.97 a (155)
MB	11.44	(124)	2.07abcd	(140)	10.25 ab (145)
USDA110	12.22	(133)	2.28 ab	(154)	10.62 a (150)
THA7	12.22	(133)	2.33 ab	(157)	10.89 a (154)
N	9.34	(101)	1.53 de	(103)	7.93 cd (112)
EA+N	10.00	(108)	1.61 cde	(109)	9.30 abc (132)
EA+MA	11.22	(122)	1.93 abcde	(130)	9.69 abc (137)
EA+MB	10.44	(113)	1.77 bcde	(120)	9.56 abc (135)
EA+ USDA110	11.89	(129)	2.19 abc	(148)	10.60 a (150)
EA+ THA7	11.33	(123)	2.08 abcd	(141)	10.06 ab (142)
F- test	NS		*		*
CV (%)	17.26		17.78		12.74

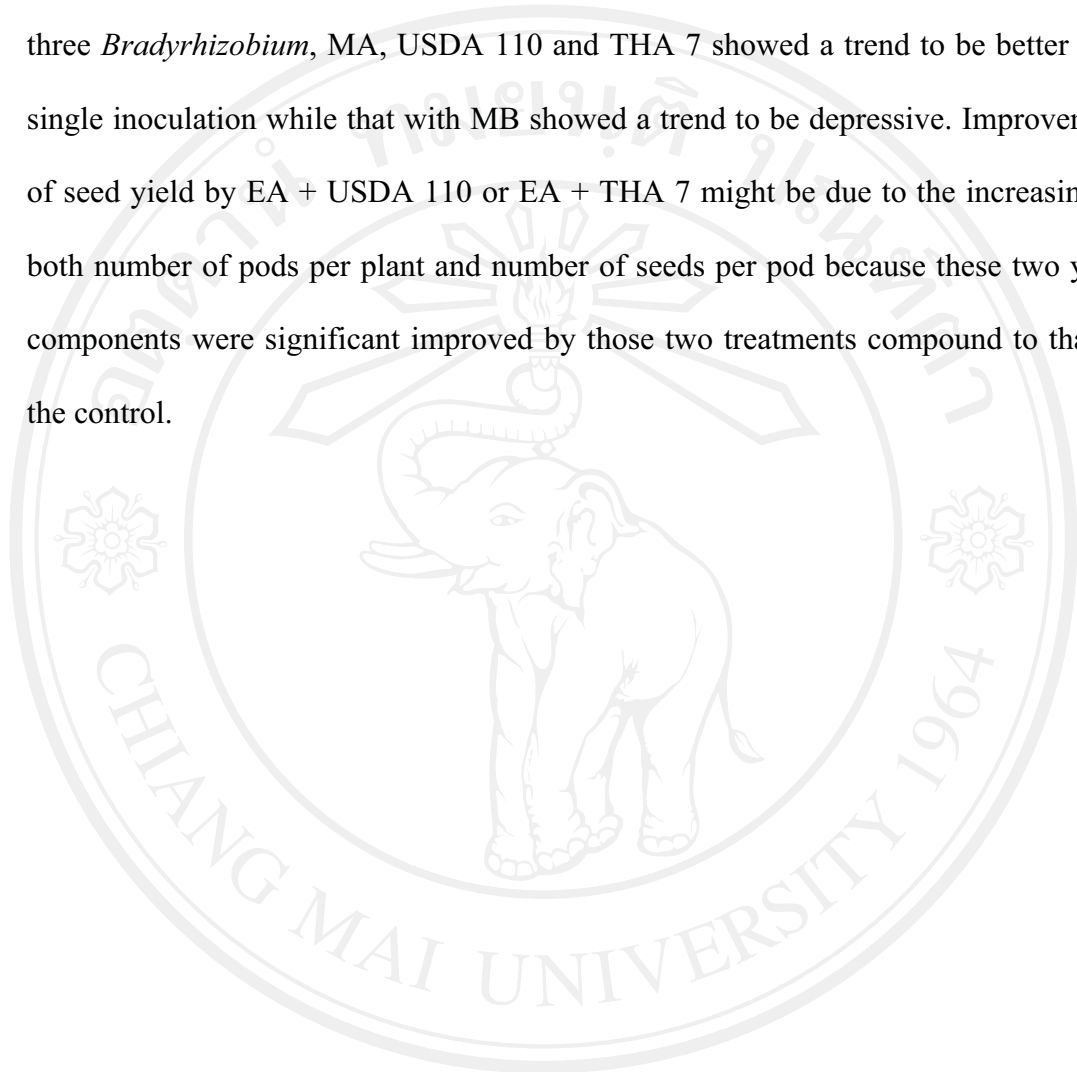
⁽¹⁾ Means of 3 replications;

NS = Non-significant; * = Significant at P<0.05

⁽²⁾ Figures in parenthesis were relative yield as compared to the control

were significantly better than the control with yield increment about 67%-80% over that of the control. Improvement of seed yield by these three bradyrhizobial

treatments were due to the increasing of the number of pod per plant particularly in MB and USDA 110 inoculated treatment. Dual inoculation of EA and each of the three *Bradyrhizobium*, MA, USDA 110 and THA 7 showed a trend to be better than single inoculation while that with MB showed a trend to be depressive. Improvement of seed yield by EA + USDA 110 or EA + THA 7 might be due to the increasing of both number of pods per plant and number of seeds per pod because these two yield components were significant improved by those two treatments compound to that of the control.



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