SYMBIOTIC NITROGEN FIXING EFFICIENCY OF DIFFERENT RHIZOBIAL STRAINS ON GRAIN AND VEGETABLE SOYBEANS AT OMON, VIETNAM

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ABSTRACT

Rhizobial strains SB83, SB174, SB177 isolated from typical soil of rice+rice+soybean cropping pattern at Phuoc Thoi village, Omon, Can Tho. In addition, standard strains SB75 and SB102 (originated from India and USA, respectively) were used to produce bio-fertilizer in powder form to study the symbiotic nitrogen fixing efficiency of different rhizobial strains on grain soybean variety Ghep Con Khuong and vegetable soybean variety VL 3 under field condition. The results on grain soybean variety Ghep Con Khuong show that among rhizobial strains, the strains SB83 and SB177 produced significantly higher number of nodules per plant, nodule dry weight / plant and bean yield than using other strains. The strains SB83 and SB177 in combination with 20 kg N / ha also performed better in symbiosis on vegetable soybean variety VL 3. These strains not only gave higher yield (over-yielded 20-31 %) as compared to control but also obtained lower nitrate content in bean grains as compared to 75 N and 150 kg N / ha applied treatments.

Key words*:* soybean, bio-fertilizer, rhizobia, nitrogen fixation

INTRODUCTION

In Mekong Delta, soybean plays an important role in rice-based farming system, soybean cultivation areas and its production in 1997 reached 14,000 ha and 28,100 tonnes, respectively (GSO 1997). Being a leguminous crop, soybean exhibits an important characteristic of fixing atmospheric nitrogen through symbiotic association with the bacterium, in its root nodules. Till 1982, the bacteria which nodulate soybean were known as *Rhizobium japonicum*. However, the slow growing bacteria which form effective N_2 fixing nodules on the roots of soybeans are presented classified as *Bradyrhizobium japonicum* (Jordan 1982) and *Bradyrhizobium elkanii* (Kuykendall et al. 1992) and fast growing rhizobia which nodulate primitive cultivars from China are grouped under *Rhizobium fredii* (Scholla and Elkan 1984). Most of the nitrogen in nature are cycled through this symbiotic process. Hence, this symbiotic relationship addresses great significance to agriculture.

The amount of nitrogen fixed varies with rhizobial strains, host cultivars and environmental conditions. When soybean seeds were inoculated with different rhizobial strains then planted in rice field, significant differences in yield have been observed by serveral workers (Subba Rao and Balasumdaram, 1971, Duong et al. 1984, Howle et al. 1987, Onder and Akcin 1991). Here, we introduce the preliminary results on the symbiotic nitrogen fixing efficiency of

different rhizobial strains on soybeans under Phuoc Thoi and CLRRI's field conditions.

MATERIALS AND METHODS

Rhizobia cultures and inoculant*:* The origin and characteristics of five rhizobial strains are presented in Table 1. SB75, SB83, SB102, SB174, SB177 which were used in this experiment were prepared in powder form at CLRRI's Microbiology Lab:

- Charcoal plus soil carrier based inoculants for these about strains of rhizobia (Jauhri et al. 1970)
- Each inoculant carried around 3.6 – 9.0 x 10^8 rhizobial cells per gram, enumerated by plate count method (Vincent 1970).

Soybean cultivar*:* Grain soybean variety "Ghep Con Khuong" was locally cultivated in Can Tho province and vegeTable soybean variety "VL3" was obtained from CAFATEX company, Can Tho city, Vietnam.

Experiment 1: "Symbiotic efficiency on grain soybean variety Ghep Con Khuong":

A field experiment was conducted in 1998-1999 dry season at the CLRRI experimental farm. Ten treatments were listed as followed: uninoculated control, SB75, SB83, SB102, SB174, SB177 and 40N, 60N, 80N, 120N. Eight rows in each plot were spaced at 40 cm apart. Ten holes / row, with 4-5 seeds / hole were spaced at 20 cm apart. Chemical fertilizer applications were proceeded as followed: single supper phosphate 40 kg P_2O_5 / ha applied as the basal dose in all plots, 20 % N applied at 7 days after sowing (DAS), 50% N at 20 DAS and 30% N at 30 DAS. Rhizobial inoculant treatments were inoculated: 50 g of rhizobial inoculant were mixed with 500 g of seeds after applying 20 ml of 10% sucrose solution for 30 minutes then sowing . At 45 days after sowing, five plants in each plot were uprooted to evaluate the symbiotic characteristics. After maturity the crop was harvested and bean yield (t/ha) was calculated.

Experiment 2: "Symbiotic efficiency on vegeTable soybean variety VL3".

A field experiment was conducted in 1998-1999's dry season at the CLRRI experimental farm. Six treatments were listed as followed: uninoculated control, SB83 + 20N, SB174 + 20N, SB177 + 20N, 75N alone, 150N alone. Eight rows in each plot were spaced at 40 cm apart. Ten holes / row, with 4-5 seeds / hole were spaced at 20 cm apart. Chemical fertilizer applications were proceeded as followed: single supper phosphate 40 kg P_2O_5 / ha applied as the basal dose in all plots, 20 % N applied at 7 days after sowing (DAS), 50% N at 20 DAS and 30% N at 30 DAS. Rhizobial inoculant in combination with 20 N treatments were applied: 50 g of rhizobial inoculant were mixed with 500 g of seeds after applying 20 ml of 10% sucrose solution for 30 minutes then sowing. At 10 DAS, 20kg N / ha was applied. At 45 DAS, five plants in each plot were uprooted to evaluate the symbiotic characteristics. At 60 DAS, the crop was harvested and bean yield (t /ha) was calculated.

The two experiments were designed in randomized block with three replications. The data were statistically analysed.

Table 1. The origin and physiological characteristics of five rhizobial strains (Man 1994)

Culture No.	Rhizobial strain	Origin	Physiological characteristics			
			Fast/ slow rhizobia	% NaCl tolerance (W / V)	N-ase activity u. M. C_2H_4 /hr/culture	I. A. A Production (mg / ml)
SB 75	*Rhizobium fredii*	India	Fast (48 hr.)	3.0	0.000	5.35
SB 102	*Bradyrhizobium japonicum*	USA	Slow (> 48 hr.)	1.5	0.851	4.75
SB 83	*Rhizobium fredii*	Vietnam	Fast (48 hr.)	3.0	0.000	2.20
SB 174	*Bradyrhizobium sp*	Vietnam	Slow (> 48 hr.)	1.5	1.662	4.15
SB 177	*Rhizobium Fredii*	Vietnam	Fast (48 hr.)	2.5	0.035	7.05

Note: SB 83, SB 174, SB 177 isolated from typical soils of rice - rice - soybean system, at Phuoc Thoi village, Omon Dist., Can Tho Province, Mekong Delta.

RESULTS AND DISCUSSION

Inoculation with suiTable strains of *Rhizobium japonicum* is recognised to be essential for increasing the final yield and quality of legumes (Arruda et al. 196). The symbiotic nitrogen fixing efficiency of different rhizobial strains were compared with different doses of nitrogen application in grain soybean variety Ghep Con Khuong presented in Table 2. The number of nodules per plant indicated that there were non-significant differences in number of nodules/plant among rhizobial strains SB83, SB174 and SB177. However, these strains exhibited significantly higher numbers of nodules than in control, rhizobial strains SB75, SB102 and treatments in which 40 N, 60 N, 80 N, 120 N were applied.

The data on dry weight of nodules also showed that the highest value (mg / plant) was obtained in SB 177 treatment, then SB 83. The lowest value was recorded in 80 N and 120 N treatments.

The results on number of nodules/ plant and dry weight of nodules / plant were reported by many workers (Gibson and Harper 1985, Tilak 1991) that high dose of nitrogen fertilizers decreased the number of of nodules/ plant and dry

weight of nodules / plant. On the contrary, 80N and 120N treatments exhibited significantly higher plant height and dry weight of biomass (T/ ha) than in the treatments of rhizobial strains SB 75, SB 83, SB 102, SB 174 and SB 177.

Table 3 indicated that there were non-significant differences in 100-grain weight and number of seeds / pod among treatments. But these treatments 40 N, 60 N, 80 N, 120 N, SB 75, SB 83, SB 102, SB 174 and SB 177 significantly differed from control in higher number of pods/plant.

Treatments SB83 and SB177 exhibited significantly higher bean yield than SB75, SB102, SB174 and control. Dry weight of nodules / plant and number of nodules / plant were more closely related to seed yield of soybean (Khurana et al. 1984).

Those strains SB83, SB174 and SB177 which isolated from Mekong Delta were found to possibly perform symbiotic characteristics and to obtain better bean yield than the standard strains SB75 and SB102 under soil conditions of Mekong Delta. Most effective *Bradyrhizobium japonicum* strains which similarly result in significant increases in soybean yield and uptake of N through fixation under Thailand field conditions, were isolated from Thailand soils (Kucey et al. 1988).

The symbiotic nitrogen fixing efficiency of different rhizobial strains were compared with different doses of nitrogen application on vegetable soybean variety "VL 3" presented in Table 4.

Non-significant differences in dry weight of nodules (mg/ plant) and 100-grain weight among treatments were recorded. However, the treatments SB174 + 20 N and SB177 + 20 N exhibited significantly higher number of nodules/ plant than the other treatments.

Otherwise, treatments 150 N and 75 N gave significantly higher plant height than the others, treatment 150 N and 75 N also found to be higher number of pods/ plant than the others.

Although there were non-significant differences in yield of fresh grains among treatments, 150 N and 75 N application gained significantly higher yield of fresh pods as compared to others.

Table 5 also indicated that all treatments inoculated with rhizobial inoculant in combination with 20 N exhibited lower of nitrate content in grains as compared to 150 N and 75 kg N / ha alone. This is very important information to convince our farmers apply rhizobial inoculant. Minimum NO_3 content of grains was recorded in treatment SB177 + 20 N, then SB174 + 20 N, and SB83 + 20N. Similarly, Ngau and Hiep (1999) inoculated effective USDA 110 ^{SB} on vegetable soybean VL 3, the NO_3 content of grains was found to be the lowest as compared to treatment 100 kg N/ ha alone.

Table 2. Symbiotic efficiency on grain soybean variety "Ghep Con Khuong" under CLRRI's field conditions. (Mean values of three replicates at 1998-1999 dry season)

Treatment	Number of nodules / plant	Dry weight of Nodules /plant	Plant height (cm)	Yield of biomass (T/ ha)
1) Control	54.2	367.9	42.0	5.35
2) 40N	56.3	414.0	51.5*	6.89
3) 60N	57.3	230.4	54.5*	8.12*
4) 80N	38.2	225.0	57.8*	8.57*
5) 100N	38.0	206.3	58.1*	8.73*
6) SB83	90.1*	636.7*	45.7	8.30
7) SB75	65.0	435.3	43.9	6.65
8) SB177	80.0*	645.0*	46.3	6.73
9) SB174	64.7	518.3	43.4	5.71
10) SB102	76.4*	480.3	45.7	6.72
CV (%)	18.0	23.5	10.4	15.8
LSD (0.05)	19.1	167.7	7.7	1.93

Table 3. Symbiotic efficiency on grain soybean variety "Ghep Con Khuong" (Mean values of three replicates, 1998-1999 dry season)

Treatment	Number of pods / plant	100-grains weight (g)	Number of seeds / pod	Grain yield (T / ha)
1) Control	14.3	27.3	1.73	1.44
2) 40 N	25.3*	28.2	1.69	1.87*
3) 60 N	29.5*	28.7	1.71	2.11*
4) 80 N	31.1*	29.0	1.74	2.50*
5) 100 N	28.8*	30.0	1.86	2.45*
6) SB83	26.8*	28.9	1.82	2.09*
7) SB75	25.7*	27.5	1.66	1.55
8) SB177	28.1*	29.4	1.84	1.92*
9) SB174	23.8*	27.3	1.81	1.51
10) SB102	22.7*	28.7	1.67	1.65
CV (%)	17.2	4.9	7.5	11.9
LSD (0.05)	7.53	NS	NS	0.39

Table 4. Symbiotic efficiency on vegetable soybean variety "V L 3" under field conditions. (Mean values of three replicates, 1998-1999 dry season)

Treatment	Number of nodules / plant	Dry weight of nodules (mg/ plant)	Height of plant (cm)	Number of Pods / plant	100-grains weight (g)
1) Control	33.5	560	33.4	21.1	48.3
2) 150 N	30.2	417	67.7*	32.2*	50.1
3) 75 N	36.1	513	51.0*	28.0*	51.2
4) SB83+ 20 N	43.2	767	33.9	24.1	51.4
5) SB177+ 20 N	47.7*	803	34.6	24.6	49.1
6) SB174+ 20 N	51.5*	767	34.7	26.5*	50.2
CV (%)	13.8	24.1	14.6	14.4	10.6
LSD (0.05)	10.15	NS	11.2	4.80	NS

Table 5. Symbiotic efficiency on vegetable soybean variety "V L 3" under field conditions (Mean values of three replicates,1998-1999 dry season)

Treatment	Yield of fresh pods (T / ha)	Yield of fresh grains (T / ha)	NO_3 content of grains (mg / kg)	Yield increase over control (%)
1) Control	5.89	3.42	333.5	-
2) 150 kg N/ha	11.53*	5.87	1009.2	95.7
3) 75 kg N/ha	8.28*	5.26	981.5	40.5
4) SB83 + 20 N	7.05	4.16	667.0	19.7
5) SB177 + 20 N	7.35	4.15	326.2	24.8
6) SB174 + 20 N	7.72	4.26	335.4	31.1
CV (%)	14.3	21.4	-	-
LSD (0.05)	2.07	NS	-	-

CONCLUSIONS

- Among rhizobial strains, SB83 and SB177 were found to perform better symbiotic characteristics on grain soybean variety " Ghep Con Khuong than

the other strains. These strains produced the highest number of nodules / plant, dry weight of nodules / plant and obtained higher bean yield than strain SB174, SB75 and SB102.

- The strains SB83 and SB177 were also found to perform better symbiotic characteristics on vegetable soybean variety "VL 3". These strains not only gained higher yield (over-yielded to check as 20-31 %) but also lowered NO_3 content in bean grains as compared to 75 N and 150 kg N / ha alone.

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TÓM TẮT

Hiệu lực cố định đạm cộng sinh của các dòng vi khuẩn *Rhizobia* trên đậu nành địa phương và đậu nành rau

Những dòng vi khuẩn *Rhizobia* SB 83, SB 174, SB 177 được phân lập từ đất của hệ thống canh tác hai vụ lúa - đậu nành thuộc xã Phước Thới, huyện ÔMôn, tỉnh Cần Thơ và hai dòng chuẩn SB 75 và SB 102 (có nguồn gốc từ Ấn Độ và USA) được sử dụng để điều chế phân sinh học ở dạng bột nhằm nghiên cứu hiệu lực cố định đạm cộng sinh của các dòng vi khuẩn *Rhizobia* này trên giống đậu nành địa phương "Ghép Cồn Khương" và đậu nành rau ở điều kiện ngoài đồng. Kết qủa trên giống đậu nành địa phương" Ghép Cồn Khương "cho thấy rằng trong các dòng vi khuẩn *Rhizobia*, hai dòng SB 83 and SB 177 tạo ra số nốt sần/cây, trọng lượng khô của nốt sần/cây cao và năng suất đậu cao hơn so với các dòng vi khuẩn khác một cách có ý nghĩa thống kê. Hai dòng SB 83 và SB 177 khi được ứng dụng bón phối hợp với 20 N (kg/ha) cũng có biểu hiện cộng sinh tốt trên giống đậu nành rau "VL 3". Hai dòng này không những cho năng suất cao hơn (20 - 31 %) so với đối chứng mà còn giảm hàm lượng NO_3 trong hạt khi so sánh với mức bón 75 N và 150 kg N/ha riêng lẽ.