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Increase in root nodulation and crop yield of soybean by native *Bradyrhizobium japonicum* strains

Namraj Dhami[1*] and Braj Nandan Prasad[2]

*[1]The School of Pharmaceutical and Biomedical Sciences, Pokhara University, Pokhara, Nepal; [2]Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal; *Corresponding author, e-mail: nrdhami@gmail.com*

Abstract

Native strains of *Bradyrhizobium japonicum* were tested for their effectiveness on nodulation, crop yield and nitrogen fixation in soybean (*Glycine max*). *B. japonicum* strains were isolated from soybean root nodules collected from different agro-climatic regions of Far Western Nepal, *viz*. Dipayal (607 m asl), Dadeldhura (1097 m asl), Silgadhi (1209 m asl) and Bajura (1524 m asl). The strains were characterized by studying colony characteristics, growth response with Congo red and Bromothymol blue, and Gram staining. The native bradyrhizobial strains were authenticated by performing infection test on soybean seedlings. All the four strains were found compatible and effective on root nodulation, crop yield and soil nitrogen (N) content. Inoculation of these strains increased soybean root nodulation by 247-343% and crop yield by 45-204%. There was strong positive correlation (r = 0.982) between number of root nodules and crop yield, which suggest that optimization of root nodulation by inoculating compatible and effective *B. japonicum* strains significantly increase the soybean crop yield. Soil N content of inoculated experimental pots was increased by 13-33%. However, variability among different strains was observed in their effect on root nodulation and yield performance. *B. japonicum* strain collected from Silgadhi was found to be the most effective in increasing nodule number and crop yield by 343% and 204% respectively.

Key-words: *Glycine max*, gram staining, inoculation, nitrogen fixation.

Introduction

Soybean [*Glycine max* (L.) Merr.] is highly nutritional protein supplement and is used in the form of cooking oil or snacks items, as well as in the form of soya sauce, soya milk, etc. The protein content in soybean has been found up to 43% of its dry weight. Soybean production has increased by 10 folds (from 17 million tones in1950 to 179 million tones in 2002) in the last 50 years. Soybean is one of the important legume crops in Nepal, where it is grown covering about 22,073 ha area with annual production of 19,362 tones (FAO 2004). The yield potential of soybean in mid hills of Nepal is 4.5 t/ha (NARC 2002), while average national yield is only 0.877 t/ha (FAO 2004).

One of the important factors affecting soybean production is the effectiveness of native bradyrhizobia existing in the soil. The bradyrhizobia present in all types of soil may not always be sufficient and efficient for providing adequate nitrogen to the growing soybean under existing conditions. Generally, the soil under continuous soybean cultivation is found to have higher bradyrhizobial populations, whereas the soil with little or no past history of soybean cultivation does not contain sufficient bradyhizobial populations and even if present most of the bradyrhizobia from soybean-free soil have been found to be non-symbiotic (Pongslip *et al*. 2002). The symbiotic efficiency and competitiveness of bradyrhizobia determine the yield performance of soybean. In Nepalese perspective, lack of effective bradyrhizobial population is the main obstacle for soybean cultivation (Pant and Prasad 2004). Thus, it is imperative to screen the most efficient and competent bradyrhizobia for inoculation purpose for soybean cultivation. In the present work, the effect of native bradyrhizobial strains on root nodulation and crop yield of soybean and nitrogen (N) content of soil was studied.

Materials and Methods

ISOLATION OF *BRADYRHIZOBIUM JAPONICUM* ISOLATES

Native *B. japonicum* strains were isolated from effective soybean root nodules collected from four different agro-climatic regions of far-western Nepal representing different altitudinal ranges, *viz*. Dipayal (DPL, 607 m asl), Dadeldhura (DDL, 1097 m asl), Silgadhi (SIL, 1209 m asl) and Bajura (BJR, 1524 m asl). Bradyrhizobial strains were cultured, isolated and purified by using Yeast Extract Mannitol Agar (YMA) medium (Vincent 1970), and Congo red (Somasegaran and Hoben 1994). Purified cultures were later stored at 4 ± 1^0C in YMA slants.

CHARACTERIZATION AND AUTHENTICATION OF THE ISOLATES

The native bradyrhizobial isolates were characterized by studying their presumptive characteristics, such as colony characteristics, culture response on Congo red, Bromothymol blue and Gram staining. Furthermore, the isolates were authenticated by performing infection test on sterile sand using Jensen's N-free medium and Yeast Extract Mannitol Broth (YMB) of bradyrhizobial isolates (Somasegaran and Hoben 1994; Arroyo *et al*. 1998). The inoculated and uninoculated soybean seedlings were examined for nodulation after three weeks.

POT EXPERIMENT

Pots of 22 cm height and 24 cm diameter were swabbed with absolute alcohol before 24 hours and filled with 2:1 garden soil and sterilized sand. Surface sterilized seeds of Sathiya cultivar of soybean (*Glycine max*) were sown in the pots. YMB (15 ml) of different native bradyrhizobial isolates with 4 × 107 CFU (colony forming units) was inoculated in pots (one isolate in one set of experimental pots), maintaining four replicates for each set and one set was kept uninoculated. After one week, soybean seedlings were thinned and only four healthy seedlings were maintained in each pot. Adequate water was provided to the growing plants until harvesting. Number of nodules and nodule dry matter content of inoculated and uninoculated soybean plants were recorded on 60th day after seed sowing. The yield parameters, such as number of pods, seed biomass (weight of 100 seeds) and total yield of soybean were also recorded. The nitrogen content of soil samples collected before soybean sowing and after soybean harvesting was estimated by Kjeldahl method (Bergersen 1980).

Results

Native bradyrhizobial strains were successfully isolated from effective soybean root nodules collected from different sites of far-western Nepal. Translucent creamy and raised bradyrhizobial colonies of 1.5 to 2 mm diameter were obtained after 7 days of culture in YMA plates. The bacterial colonies did not absorb any red color of Congo red in dark culture that facilitated the isolation and purification of bradyrhizobia. Furthermore, we observed alkali production during culture and gram negative responses of the isolates. The isolates were confirmed to be the *Bradyrhizobium japonicum* strains as they all produced effective nodules on soybean seedlings grown on sterilized sand. Thus, isolates, DPL, DDL, SIL and BJR, were authenticated as *B. japonicum* strains.

Inoculation of all four native *B. japonicum* strains increased root nodulation and crop yield (Tables 1 and 2). Inoculation increased the yield two to three times over uninoculated plants. We found significant positive correlations between nodule number and crop yield ($r = 0.982$, $p = 0.01$; Table 3). In inoculated plants, the number of nodules increased by 247 to 343% (Table 2). Nodule dry matter and other parameters (number of pods and seed biomass) were also increased in inoculated plants (Tables 1 and 2).

The soil N content was increased up to 33.37% after soybean harvesting (Table 4). The amount of N addition to the soil was correlated with the number of nodules ($p < 0.05$). The experimental sets in which plants were with higher number of nodules were found to have more N in the soil.

Although all the four strains were found to be efficient for root nodulation and all of them significantly increased the crop yield, there was strong variation in the degree of effect on these parameters among the four strains. *B. japonicum* strain 'SIL' was found to be the most efficient strain (Tables 1 and 2).

Table 1. Effect of *Bradyrhizobium japonicum* strains on nodulation and growth parameters in soybean after 60 days of sowing.

B. japonicum strains	NN/pl.	NDM/pl. (g)	POD/pl.	100s (g)	Yield/pl. (g)
Control	35.75	0.19	32.37	17.36	6.67
DPL	124.25	0.34	45.62	19.94	14.02
DDL	133.25	0.36	43.50	21.49	16.38
SIL	158.50	0.49	45.25	25.08	20.27
BJR	137.75	0.41	43.62	22.33	17.20

Strains: DPL–Dipayal, DDL–Dadeldhura, SIL–Silgadhi, BJR–Bajura. Growth and yield parameters: NN/pl.–nodule number per plant, NDM/pl.–nodule dry matter per plant, POD/pl.–number of pods per plant, 100s–weight of 100 seeds.

Table 2. Percent increase in nodulation and growth parameters in *bradyrhizobium*-inoculated soybean over uninoculated plants.

B. japonicum strains	Percent increase in				
	NN/pl.	NDM/pl.	POD/pl.	100s	Yield/pl.
DPL	247.55	78.94	40.93	14.86	110.19
DDL	272.72	89.47	34.58	23.79	145.57
SIL	343.35	157.89	39.78	44.47	203.89
BJR	285.31	115.78	34.75	28.62	157.87

Abbreviations as in Table 1.

Table 3. Relationships between nodulation and soybean growth and yield parameters.

	NN	NDM	POD
NDM	0.957*		
POD	0.955*	0.852*	
Yield	0.982**	0.987**	0.882*

*Correlation is significant at the 0.05 level (2 tailed); **Correlation is significant at the 0.01 level (2 tailed).
NN–nodule number, NDM–nodule dry matter, POD–number of pod.

Table 4. Nitrogen (N) addition to the soil by native *Bradyrhizobium japonicum* strains.

SN	Soil samples	N content of soil (%)	Increase in N content (%)
1	sbss	0.060	
2	sash CTL	0.067	11.67
3	sash DPL	0.068	13.34
4	sash DDL	0.071	18.34
5	sash SIL	0.080*	33.37*
6	sash BJR	0.073	21.67

sbss = soil before soybean sowing, sash = soil after soybean harvesting, *shows the best performance

Discussion

Bradyrhizobial strains were isolated from soybean root nodules. The longer duration of colony development showed slow growing nature of bradyrhizobia. Somasegaran and Hoben (1994) also suggested the requirement of 7-10 days incubation for bradyrhizobia. Alkali production during culture and gram negative responses supported the characterization of bradyrhizobial isolates.

Inoculation of all four native *B. japonicum* strains increased root nodulation and crop yield. High degree of correlation between nodule number and crop yield (Table 3) suggested that the optimization of nodulation play crucial role in increasing soybean yield. Due to the lack of sufficient *B. japonicum* cells in the soil, plants were having less root nodules in uninoculated sets, whereas in inoculated plants, the number of nodules increased by 247 to 343% over uninoculated plants. Nodule dry matter also increased with the increase in nodule number. Larger bradyrhizobial population infected more root hairs enhancing the nodule number, ultimately contributing to the higher dry matter of nodules per plant. Several studies also reported significant increase in soybean growth parameters and yield due to the inoculation of bradyrhizobial isolates (e.g., Purcino *et al.* 2000; Okereke *et al.* 2001; Pant and Prasad 2004). Okereke *et al.* (2001) observed 71 to 486% increment in nodule number due to the inoculation of *B. japonicum* isolates on soybean, which is comparable with the present finding.

The plants having higher number of nodules were found to possess high nodule dry matter and better crop yield than uninoculated plants. It indicates that the degree of root nodulation determines the crop yield in soybean. Sato *et al.* (1999) suggested that optimizing the nodulation could maximize the nitrogen fixation. According to Nelson *et al.* (1984), rate of nitrogen fixation in root nodules determines the overall performance of soybean growth, development and yield. The soil N content was increased up to 33.37% after soybean harvesting. The amount of N addition to the soil was correlated with the number of nodules. The increase in soil N content after soybean harvesting is thus due to degradation of senescent root nodules.

The results of this study suggest that inoculation of effective *B. japonicum* strain invariably increases root nodulation in soybean that in turn increases crop yield. The differences in the nodulation and crop yield parameters reveal the variability in the efficiency of native *B. japonicum* strains. Such variability in effectiveness of different rhizobial strains was also reported from other regions (e.g., Arroyo *et al.* 1998; Minamisawa *et al.* 1999; Nuntagij 1999). In the present study, the *B. japonicum* strain 'SIL' was found to be the most efficient strain in producing such effects. It is emphasized that nitorgen fixing efficiency may vary among different strains of *B. japonicum.* Therefore, inoculation of compatible and effective *B. japonicum* strain is necessary to optimize the soybean root nodulation that significantly increases the crop yield and improves the soil N status. The addition of N increases soil fertility that in turn plays an important role for subsequent crops. Thus, the use of effective bradyrhizobial isolates as biofertilizer could be the highly sustainable practice for soybean cultivation as well as to reduce the increasing demand of chemical fertilizers.

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