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Full Length Research Paper

Identification of pH tolerant *Bradyrhizobium japonicum* strains and their symbiotic effectiveness in soybean [*Glycine max* (L.) Merr.] in low nutrient soil

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Eight strains of *Bradyrhizobium japonicum* were isolated from the root nodules of soybean cultivar PK 472 collected from Adaptive Trial Centre, Bundi, India. All the isolates were authenticated through plant assay test in germination pouches. Growth of the isolated strains on different pH levels was observed and three strains namely Bj-3, Bj-5 and Bj-8 were found to be able to grow up to pH 8.5. Symbiotic efficiency of these isolates was determined under the mist house conditions in the sterilized sandy soil (pH 8.4). Maximum and minimum nodulation and vegetative growth were observed in Bj-3 and Bj-2 inoculated soybean plants, respectively. Three pH tolerant strains could also pose better results in the efficiency determination experiment. Considerable variability in terms of the symbiotic effectiveness was found in the tested strains.

Key words: Identification, symbiotic effectiveness, *Bradyrhizobium japonicum*, pH, soybean, nutrient, soil.

INTRODUCTION

The beneficial effect of *Rhizobium* and *Bradyrhizobium* in legume in terms of biological nitrogen fixation has been a main focus in the recent past (Deshwal et al., 2003), as it is an important aspect of sustainable and environmental friendly food production and long term crop productivity. Soybean is a crop that is used for human food and livestock feed. Plant improvement generally seeks to increase the proportion of dry matter production that goes to seed production (Lukiwati and Simanungkalit, 2002). *Bradyrhizobium japonicum* strains form nitrogen-fixing root nodule symbioses with soybean (Madrzak et al., 1995).

Inoculation with highly effective rhizobia, a common practice in agricultural production (Catroux et al., 2001), requires survival and establishment of inoculated rhizobia in the soil environment (Da and Deng, 2003). In Rajasthan, soybean is mainly cultivated in the south eastern part of the state covering Kota, Bundi, Baran and Jhalawar districts which is known as Haroti region. The soil of this region is vertisol and is relatively high in nutrients. However, to initiate the soybean cultivation in other areas where the soil is sandy or sandy loam with low nutrients, it is essential to obtain some effective *B. japonicum* strains having better adaptability to survive in such soil conditions. Keeping above views in the mind, the present investigation was made and the symbiotic effectiveness of some pH tolerant *B. japonicum* strains has been evaluated in low nutrient sandy soil.

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MATERIALS AND METHODS

Isolation and authentication of *B. japonicum* strains from field grown soybean

Isolates of *B. japonicum* were obtained from root nodules of field grown soybean plants (cv. PK 472) collected from Adaptive Trial Center (ATC), Bundi (Rajasthan). Serial dilution agar plate method as described in Somasegaran and Hoben (1994) was employed with 20E medium (Werner et al., 1975), for the purpose of isolation. Isolates were streak purified on the same medium and the purified

Table 1. Effect of *B. japonicum* on nodulation and vegetative growth of soybean cultivar PK 472.

Treatments	Total no. of nodules	Dry wt. of nodules (g)	Shoot dry wt. (g)	Shoot length (cm)	Root length (cm)
Control	0.00	0.00	2.31±0.11	23.00±2.01	19.05 ±1.06
Bj-1	20.83^{d}±2.28	0.11^{c}±0.02	2.77^{a}±0.18	28.08^{c}±3.11	21.00^{a}±1.00
Bj-2	19.45^{d}±1.69	0.07^{d}±0.02	2.51^{b}±0.07	26.90^{c}±1.05	21.18^{a}±2.09
Bj-3	36.90^{a}±1.70	0.18^{a}±0.01	3.23^{a}±0.15	38.33^{a}±1.11	22.00^{a}±1.51
Bj-4	20.41^{d}±1.08	0.09^{b}±0.03	2.58^{b}±0.29	27.00^{c}±0.98	19.25^{b}±1.32
Bj-5	32.66^{b}±1.32	0.19^{a}±0.01	3.02^{a}±0.05	32.27^{b}±1.41	21.90^{a}±0.78
Bj-6	25.54^{c}±1.41	0.15^{b}±0.03	2.89^{a}±0.14	31.05^{b}±2.45	19.58^{b}±0.99
Bj-7	19.90^{d}±2.01	0.10^{c}±0.01	2.39^{b}±0.10	30.00^{b}±1.69	20.63^{a}±1.01
Bj-8	29.00^{b}±2.12	0.16^{a}±0.04	2.97^{a}±0.09	31.81^{b}±1.31	19.00^{b}±1.31

Data are means of five replicates ± SE.
Values without common letters differ significantly at LSD $P<0.05$.

isolates were subjected to authentication test in growth pouches (procured from Mega International, USA) under controlled environmental conditions (day length, 14 h; temperature, 28±1°C, light intensity, 12000 lux; humidity, 70-80%). Authenticated strains were maintained as frozen glycerol stocks at -40°C.

pH tolerance study of *B. japonicum* strains

Authenticated *B. japonicum* strains were grown in the 20E broth with different pH ranging from 5.00 - 9.00. The optical density at 540 nm was recorded by spectrophotometer after 96 h of incubation.

Symbiotic efficiency of *B. japonicum* strains (Mist house experiment)

Soybean seeds (cv. PK 472; supplied by the Agricultural Research Station, Ummedganj, Kota) were surface sterilized (Call and Davis, 1988) and were grown in earthen pots containing sterilized sandy soil (5 kg of dry soil/pot) under mist house conditions, after the treatment with mid log phase cultures of eight *B. japonicum strains* employing slurry method as described by Vincent (1970). Each pot contained 2 plants of soybean and untreated seeds served as control. Experimental design was completely randomized with five replicates. Plants were irrigated daily and were maintained to grow till 45 days in the mist house having temperature 27 - 35°C, humidity 70 - 80% and light 15000 - 19000 lux.

Plants were harvested after 45 days of sowing and data pertaining to nodular and vegetative characters were recorded. Nitrogen content in shoots was estimated by Kjeldhal method (Jackson, 1973). Physico-chemical properties of sandy soil viz. pH, electric conductivity, organic carbon, available nitrogen, phosphate and potash were determined before experimentation, using standard protocols.

Statistical analysis of data

Analysis of variance (ANOVA) was performed on nodular and vegetative characters and least significant difference (LSD) was calculated at $P<0.05$.

RESULTS AND DISCUSSION

Ten isolates from the soybean cultivar PK 472 were obtained out of which eight had given positive results in the authentication test and remaining two were not able to infect the soybean plants in growth pouches. Bacterial strains showing positive results were used for further study and were designated as Bj-1, Bj-2, and so on (Table 1). The remaining two isolates were discarded.

Although all the tested eight bradyrhizobial strains have shown turbidity in the 20E broth having pH upto 8.5, yet a considerable variability in their pH tolerance was observed. Highest optical density at pH 8.5 was recorded for the strain Bj-3 followed by Bj-8, Bj-5, Bj-1, Bj-2, Bj-6, Bj-7 and Bj-4 (Figure 1). Physico-chemical properties of the experimental soil can be summed up as: pH 8.4, 0.36 mhos/cm electric conductivity, 0.15% organic carbon, 102 kg/ha available nitrogen, 50 kg/ha phosphate and 270 kg/ha potash.

Data on the effect of *B. japonicum* inoculation on nodulation and vegetative growth of soybean plants have been furnished in Table 1. Highest nodular frequency and nodule dry weight (per plant) were found in the plants inoculated with *B. japonicum* strain Bj-3. However inoculation of other strains viz. Bj-5, Bj-6 and Bj-8 also resulted in significantly high nodular number and nodule dry matter per plant. 3.46 to 39.83% increase in shoot dry weight compared to control was observed after the inoculation of *B. japonicum* strains. Maximum enhancement (39.83%) in shoot dry weight in comparison to the uninoculated plants was observed in the strain Bj-3 inoculated plants.

Shoot and root length was also potentiated by the inoculation of different *B. japonicum* strains (Table 1). Similarly, a significant increase over control in shoot nitrogen content was also seen, due to the bacterization of soybean plants. Maximum shoot nitrogen content per plant (2.18%) was estimated in the Bj-3 inoculated plants,

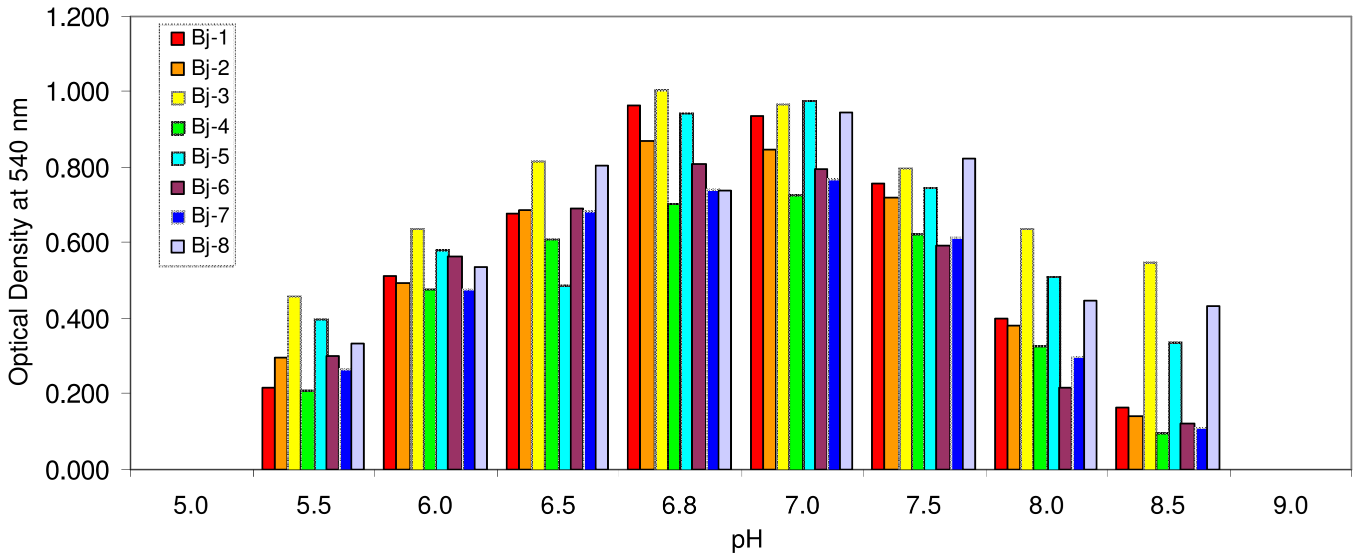

Figure 1. Growth of *B. japonicum* strains at different levels.

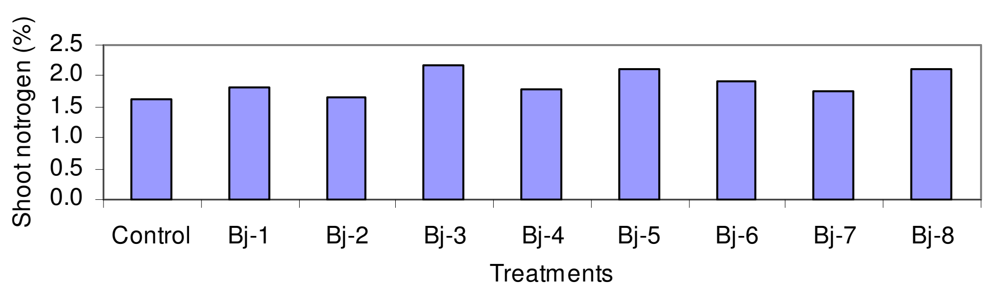

Figure 2. Effect of *B. japonicum* on nitrogen content in shoots of soybean cultivar PK 472.

which was 34.56% higher than that of the control plants (Figure 2).

Overall assessment of the present data clearly indicated that inoculation of *B. japonicum* strains enhances the nodulation, vegetative growth and N uptake in soybean plants. The symbiotic efficiency of eight isolates showed a great diversity in soybean plants and a few of them accumulated considerably high shoot dry matter as it is evident from the Table 1. These results are very much similar to those obtained by several earlier workers (Lukiwati and Simanungkalit, 2002; Zhang et al., 2002; Mahna et al., 2003).

Three *B. japonicum* strains (Bj-3, Bj-5 and BJ-8) showing high pH tolerance (Figure 1) also performed better in the efficiency determination experiment (Table1 and Figure 2) which indicated that these strains can be regarded as effective ones under low nutrient soils.

In summary, it will be logical to state that such laboratory studies are effective in identifying sensitive/resistant bacterial strains to various ecological factors, before their verification in field studies.

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