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# SHORT COMMUNICATION

# The Use of *Bradyrhizobium* to Enhance Growth and Yield of Soybean in Calcareous Soil in Uzbekistan

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# ABSTRACT

In this work the effect of inoculation with *Bradyrhizobium japonicum* S2492 on soybean (*Glycine max* (L) Merr) growth, nodulation and yield in nitrogen-deficient soil of Uzbekistan was studied. The field experiments were carried out in Tashkent Province of Uzbekistan in a randomized complete block design with four replicates of each treatment. The results revealed positive effects on growth, nodule number and yields of soybean after inoculation with *B. japonicum* S2492. The yield of soybean varieties was 48% higher for inoculated than for

uninoculated plants. The effect of the inoculation was specific for variety but not for growth type. The protein and oil contents of seeds also increased after inoculation. It was concluded that *B. japonicum* S2492 can be considered as a biofertilizer for increasing the productivity of soybean in nitrogendeficient soils in Uzbekistan.

**Key words:** Soybean; Plant growth; Nodulation; *Bardyrhizobium* 

### Introduction

As a consequence of inappropriate applications of mineral fertilizers during continuous cotton cultivation, Uzbekistan suffers from problems such as pollution of agricultural lands, water resources, and soil salinization. In an attempt to reduce these chemical inputs and raise soil quality and sustainability, new biotechnological practices, such as the application of bacterial inoculates have been investigated to improve crop production in Uzbekistan. Many countries use bacterial fertilizers such as *Rhizobium* inoculants for agricultural practices (Smith 1992; Höflich 1993; Dashti and others 1997; Groppa and others 1998). Several inoculation field experiments set up on different soil types showed a positive effect of inoculation with certain strains of *Bradyrhizobium* on crop plant growth, including soybean (Molla and others 2001; Bai and others 2002). Soybean is an important crop worldwide

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**Table 1.** The Effect of *Bradyrhizobium japonicum* S 2492 on Dry Matter Plant Height and Nodulation of Soybean Varieties

| Soybean varieties | Shoot dry matter (g. per plant) |      | Plant height (cm) |       | Nodule number (per plant) |       |
|-------------------|---------------------------------|------|-------------------|-------|---------------------------|-------|
|                   | 2000                            | 2001 | 2000              | 2001  | 2000                      | 2001  |
| Control, Orzu     | 4.0                             | 4.6  | 43.1              | 47.6  | 19.8                      | 20.2* |
| Inoculated, Orzu  | 6.2*                            | 6.7* | 48.9*             | 54.8* | 28.1*                     | 31.7* |
| Control, UZB2     | 3.8                             | 3.9  | 56.4              | 59.0  | 18.4                      | 19.4  |
| Inoculated, UZB2  | 5.2*                            | 5.8* | 61.8*             | 64.2* | 29.1*                     | 29.6* |
| Control, UZB6     | 4.2                             | 4.8  | 70.1              | 70.8  | 18.8                      | 19.2  |
| Inoculated, UZB6  | 5.7                             | 5.9* | 72.2              | 76.4  | 22.0                      | 23.4* |

(Field experiments, results from 2000 to 2001 years \*Significant differences P < 0.05)

because it is a N<sub>2</sub> fixing leguminous plant, offering high quality protein, capable of returning N<sub>2</sub> to the soil. Factors affecting inoculum success include soil temperature, soil type, soil N content and soil moisture. In tropical agriculture, the potential for improved soybean productivity from rhizobial inoculation is generally much higher than for temperate systems. Many legumes will not be nodulated by rhizobia in acid soils (Taylor and others 1991) and low soil temperature during the early growth stages is a potential limiting factor to soybean production in regions with short growing seasons (Norman 1978). Rahmani and Rastin (2001) reported an effective symbiosis between soybean and Bradyrhizobium japonicum in N-deficient soils but the effect of B. japonicum on soybean growth in N deficient calcareous soils under conditions in Uzbekistan has not been investigated. Therefore the aim of this research was to evaluate the effect of B. japonicum on plant growth and yield of soybean varieties grown in nitrogen-deficient soils of Uzbekistan.

# MATERIAL AND METHODS

The study site is located in an experimental station of the University of Agriculture of the Tashkent province in the northeastern part of Uzbekistan, and represents a continuously cultivated field (calcareous calcisol soil). The soil chemical characteristics were as follows: 1% organic matter, 0.6 mg N-100 g<sup>-1</sup> soil; 3.0 mg P-100 g<sup>-1</sup>; 12 mg K-100 g<sup>-1</sup>; 6 mg Mg-100 g<sup>-1</sup> soil. The total carbon content ( $C_{tot}$ ) was determined by the Nelson Somers method (Nelson and Somers 1975), and the total nitrogen content ( $N_{tot}$ ) was determined by the Kjeldahl method (Keeney and Nelson 1982). The molybde-

num blue method was used to determine total phosphorus content ( $P_{tot}$ ) in the soil and potassium (K) was determined using the flame photometric method (Rhiem 1985). An atomic absorption spectrophotometer was employed to measure calcium chloride ( $CaCl_2$ ) and extractable magnesium (Schachtschnabel and Heinemann 1974).

The 2-year (2000 and 2001) experiments were set up as randomized complete block designs with four replicates. Plots  $(6 \times 2.5 \text{ m})$  were divided into rows spaced 0.5 m apart and a space of 1 m was allowed between plots. The mean temperature of the growing season in 2000 and 2001 was 15-17°C (April, May) and 28-30°C (June July). Soybean seeds (Glycine Max) cv. Orzu, Uzb-2, Uzb-6 were obtained from the University of Agriculture of Tashkent. The B. japonicum strain S2492 was provided by the National Culture Collection of Microorganisms of Uzbekistan. Sterilized seeds were cultivated after coating with the peat-based inoculants of B. japonicum S2492 that had 10<sup>8</sup> cell g<sup>-1</sup> peat (10 g of peat-based inocula for 1 kg seed). Bacterial inoculants were prepared following the method described by Höflich and others (1987). Uninoculated soybean plants were used as controls. Soybean seeds were planted by hand in each plot in the beginning of April and were irrigated by furrow irrigation. Two months after sowing, the number of nodules per soybean plant was estimated. At the end of the growing season, plants were harvested to determine soybean seed yield as well as protein and oil content. Shoot and root dry weights were recorded after drying at 100°C. Protein was assayed according to Bradford (1976) using bovine serum albumin (BSA, fraction V) to standardize the assay. The essential oil content in the seeds was determined by a hydrodistillation method on a Clevenger type apparatus (Charles and Simon 1990). Statistical

**Table 2.** The effect of *Bradyrhizobium japonicum* S 2492 on Yield, Protein and Oil Content of Seeds of Soybean Varieties

| Soybean varieties | Yield (g per plant) |       | Protein content (%) |       | Oil content (%) |       |
|-------------------|---------------------|-------|---------------------|-------|-----------------|-------|
|                   | 2000                | 2001  | 2000                | 2001  | 2000            | 2001  |
| Control, Orzu     | 7.0                 | 8.4   | 35.7                | 35.9  | 17.2            | 17.9  |
| Inoculated, Orzu  | 10.8*               | 13.4* | 38.8                | 39.2  | 19.9*           | 20.4  |
| Control, UZB2     | 5.8                 | 6.2   | 32.6                | 32.9  | 17.0            | 17.5  |
| Inoculated, UZB2  | 9.1*                | 9.5*  | 40.2*               | 40.6* | 19.4*           | 20.9* |
| Control, UZB6     | 6.6                 | 7.1   | 32.1                | 32.8  | 17.6            | 18.0  |
| Inoculated, UZB6  | 9.3                 | 11.2* | 38.4*               | 39.2* | 18.7            | 19.6  |

(Field experiments, results from 2000 to 2001 years, \*Significant differences P < 0.05)

analysis was performed using ANOVA and means were calculated and their differences were tested for significance by using the Student Newman test (P < 0.05).

# RESULTS AND DISCUSSION

The results of the experiments revealed that inoculation with B. japonicum S2492 increased dry weight, height, nodulation and soybean yield. The level of increases varied among varieties. Inoculation increases shoot dry weight up to 45% compared to uninoculated plants. Inoculation increased soybean height and nodule numbers. A greater proportion of nodules developed on the main root in the Orzu soybean variety after inoculation. The Orzu variety was affected by B. japonicum S2492 inoculation more positively than other varieties (Table 1). Bacterial inoculation increased the yield in all soybean varieties (Table 2). These results could be explained by the reported symbiosis efficiency between soybean and B. japonicum S2492 strains under the low levels of available soil nitrogen (Rahmani and Rastin 2001). Zhang and others (1996) and Dashti and others (1997) have reported similar results, suggesting that inoculation of legume plants with B. japonicum increased nodule numbers, plant weight and seed yields under greenhouse and field conditions. Bai and others (2002) found that Bradyrhizobium japonicum inoculation of soybean increased nodule weight by 37%, root weight by 35%, and shoot weight by 29%, respectively.

The protein and oil contents in seeds also responded positively to inoculation with *B. japonicum* S2492. Protein content increased by 23% and oil content by 19% in both inoculated Orzu and Uzb 2 soybeans variety (Table 2). The effect of bacteria

inoculation on the UZB 6 variety was lower than that of other soybean varieties, in accordance with reports by Höflich(1993) for pea varieties inoculated with *Rhizobium*.

Bacterial stimulation of soybean growth and yield was higher in the second year of cultivation. The first year of cultivation of plants was probably affected by soil composition and fertility. These results are important because the use of *B. japonicum* S2492 as a bacterial fertilizer provides a new technological approach, that may reduce chemical fertilizers and help produce healthy foods in Uzbekistan even under N-poor soil conditions.

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