

Bioinoculation of native *Bradyrhizobium* and *Aspergillus* for assessment of productivity attributes of soybean cultivar JS-335

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ABSTRACT

For the improvement of crop plant growth and soil fertility in an ecofriendly way, bioinoculation is the best practice. Bioinoculation is available both in liquid and solid forms commercially for agriculture purpose but the native rhizobial strains provide better nitrogen fixation as compared to commercial ones. Keeping this view in mind, pot experiments were conducted to study the effect of co-inoculation of native strains of *Bradyrhizobium japonicum* and *Aspergillus niger* (plant growth promoting fungi) on yield contributing characters e.g., pods per plant, seeds per pod, dry weight of pods and seeds of soybean cultivar JS-335. This cultivar is commonly grown in the Malwa region of Madhya Pradesh state (India). Before evaluation of plant yield attributes, ten isolates were screened for their nitrogen fixing ability in terms of NOPP (Nodule Occupancy Per Plant), shoot and root length, and their dry weight. Overall, 27 pots were run parallel for the experiment. Nitrogen fixing potential results emphasized that out of ten isolates only three (B1, B2 and B3) isolates were found promising and their nitrogen fixing potential was statistically significant over controls. DMRT test was used for further statistical analysis. Results revealed that co-inoculation of indigenous *B. japonicum* and *A. niger* significantly increased the yield over the controls in all test parameters. This study also ascertain information that more emphasis be given to the native (indigenous) microbes for the preparation of inoculum rather than the commercial ones.

Keywords: *Aspergillus niger*, *Bradyrhizobium japonicum*, Potato Dextrose Broth

INTRODUCTION

Soybean is known as a 'miracle crop' and the main producer countries of soybean are the United States (32%), Brazil (28%), Argentina (21%), China (7%) and India (4%) [1]. It is cultivated in India mainly as a *kharif* crop that grows well in a warm climate with intermediate to heavy rainfall. In India, productivity of soybean has been recorded highest in Madhya Pradesh (a central part of India with Black Cotton Soil) with gross production of 75.3% with a range of temperature (27°C) for July and August [2]. Soybean-growing districts are grouped according to Yield Index [3]. The maximum production in Malwa region of Madhya Pradesh is recorded in Indore, Ujjain, Ratlam, Dhar, Dewas and Mandsaur districts. Soybean is grown in almost all parts of the Ujjain district (**Photo Plate-1**). Soybean plant forms symbiotic association with *Bradyrhizobium japonicum* (Rhizobiales order), is characterized by its ability to infect the roots of legumes and form nodules, specialized organs within which, as bacteroids, made the biological fixation of atmospheric nitrogen (N₂), or the reduction of nitrogen in ammonium (NH₄⁺), a reaction catalyzed by nitrogenase [4]. Legumes play a critical role in agriculture with fixing atmospheric N₂ in symbiosis and hence make them outstanding colonizer of reduced nitrogen [5]. Various reported works are available which support that crop productivity can be improved by the use of different inoculation techniques such as biological fertilizers [6],[7],[8]. The 'rhizosphere' is the area surrounded by the roots and characterized by the continuous supply of low molecular weight compounds exuded from roots. Due to this, microbial activity is very high in this region as compared to non-rhizospheric region. The rhizosphere supports a large metabolically active microbial population higher than in non-rhizosphere soil [9]. In this region a large number of Plant Growth Promoting Microorganisms are present which contribute to increased plant growth by increasing nitrogen uptake,

synthesis of phytohormones (auxin, cytokinin), minerals solubilization and iron chelation as well as through the solubilization of phosphate minerals [10-15].

The present study was done for the evaluation of possible role and potential of indigenous *B. japonicum* and plant growth promoting fungal isolates on plant yield in terms of number of pods per plant, number of seeds/pod and dry weight of pods and seeds.

MATERIALS AND METHODS

Plant collection and isolation of microorganisms

Soybean cultivar JS-335 was procured from Jawaharlal Nehru Agriculture Centre, Vikram Nagar Ujjain. For nodule collection, Soybean plants were excavated carefully avoiding any injury and were brought in the laboratory. Root nodules were washed under a gentle stream of water. Young, healthy and pinkish root nodules were selected for isolation [16]. For fungal isolation Johnson et al. 1959 [17] method was followed. The identification of *B. japonicum* was done using Bergey's Manual of Determinative Bacteriology 9th edition [18], while for fungi Manual of Soil Fungi [19] was followed. Total ten strains of *B. japonicum* and two strains of *A. niger* were isolated, screened, identified and used for further study for their potential to fix atmospheric nitrogen. Out of ten only three isolates namely B1, B2 and B3 along with one fungal isolate (*A. niger*) were screened for further yield potential study.

Broth culture

All isolates of *B. japonicum* were grown in the yeast extract mannitol broth for 7 days at $27\pm 2^\circ\text{C}$. *Composition of medium*: Mannitol-10 g., K_2HPO_4 -0.5 g., $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ - 0.3 g., NaCl- 0.1 g., Yeast Extract- 0.5 g., Distilled Water (D/W)- 1000 ml [20]. For the fungal isolate, Potato Dextrose Broth medium was used (HiMedia Lab.).

Pot arrangement, Planting, harvesting and processing

For seed germination and pot arrangement Sharma and Kumawat, (2012) method was followed [21].

Determination of Yield potential attributes

At the time of maturation (95 days after sowing), plants of different treatments of cultivar JS-335 were removed and for the authentication of inoculum, yield contributing attributes were taken into consideration. Further, following parameters were evaluated for the assessment of yield.

Average number of pods per plant and dry weight of pods

Total number of pods per plant were counted and average value was calculated. Pods without seeds were not taken in the counting. Seeds were removed from the pods and the weight of total pods was measured after drying them at 72°C for 24 hours.

Average number of seeds per pod and dry weight of seeds

Seeds were removed and seeds per pod were counted. For dry weight, seeds were dried in hot air oven at 72°C for 24 hours.

Statistical analysis

The statistical analyses were done using SPSS (Statistical Package, Version 10.0). The variables were subjected to ANOVA and were tested for significance while Duncan's Multiple Range Test (DMRT) was followed for the data.

RESULTS AND DISCUSSION

Nitrogen fixation is one of most important biological processes on earth and *Rhizobium* inoculation of legumes is one of the success stories of world agriculture [22]. The response of inoculum with respect to nodule formation (NOPP)/ root and shoot length and their dry weight was reflected in dry matter production as well. **Figure 1, 2, and 3** shows the effect of different *Bradyrhizobium* strains on plant growth. External inoculation increased the number of root nodules when compared to their respective controls. There is a strong positive relationship between number and dry weight of nodules and yield per plant in soybean. In our study, there was a significant difference among isolates over respective controls. However, response of co-inoculation treatment varied with different bacterial strains. Based on the nodulation, isolates were grouped as excellent (more than 21 nodules per plant), very good (16-20 nodules per plant) and good (11-15 nodules per plant) (not mentioned here).

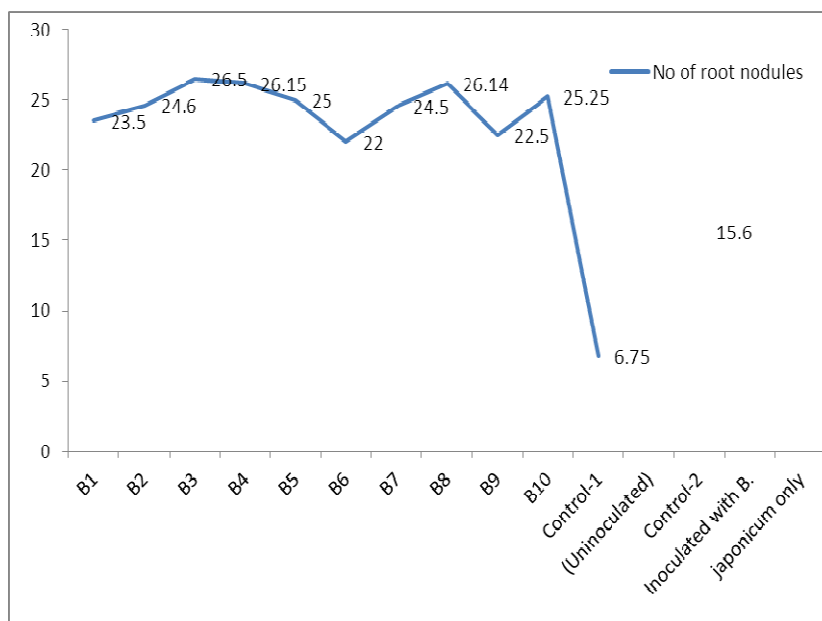


Figure:1 Total number of root nodules per plant co-inoculation of *B. japonicum* and *A. niger* Asp n1 on number of root nodules

Increase in shoot length and dry weight was significantly higher in all bacterial isolates over respective controls in combination with *A. niger*. Reasons behind such type of results might be the production of some kind of plant growth promoting phytohormones by the fungal isolate and synergistic effect with *B. japonicum* [23], [24]. IAA production have stimulatory effect on the plant growth and when crop is inoculated with the IAA producing isolates, significant increase in uptake of N, P, K, Ca and Mg has been reported [25]. Some plant growth promoting microorganisms may promote plant growth indirectly by affecting symbiotic N₂ fixation, nodulation and shoot growth [26].

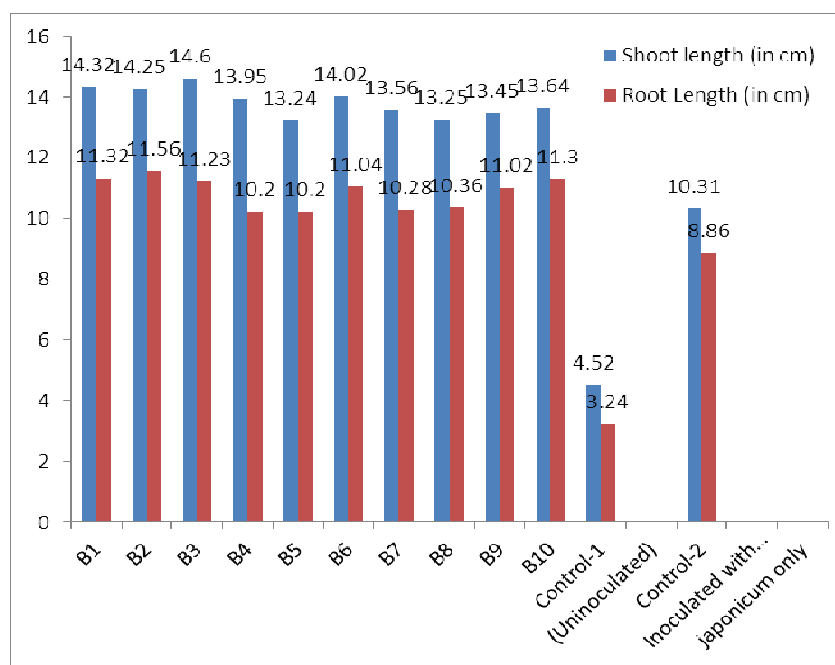


Figure:2 Shoot and root length of soybean cultivar JS335 with different treatment of Bradyrhizobial and fungal isolates

With reference to increasing international concern for food and environmental quality, the use of PGP (Plant Growth Promoting) microbes for reducing chemical inputs in agriculture is a potentially important issue. PGP microbes have been used in various crops for seed emergence, enhanced growth and crop yield [22], [27]. Use of composite biofertilizers can increase the soil fertility to a great extent. It has been proved that biofertilizers are cost effective, safe and ecofriendly alternative to chemical fertilizers.



Photoplate-1: Field of soybean

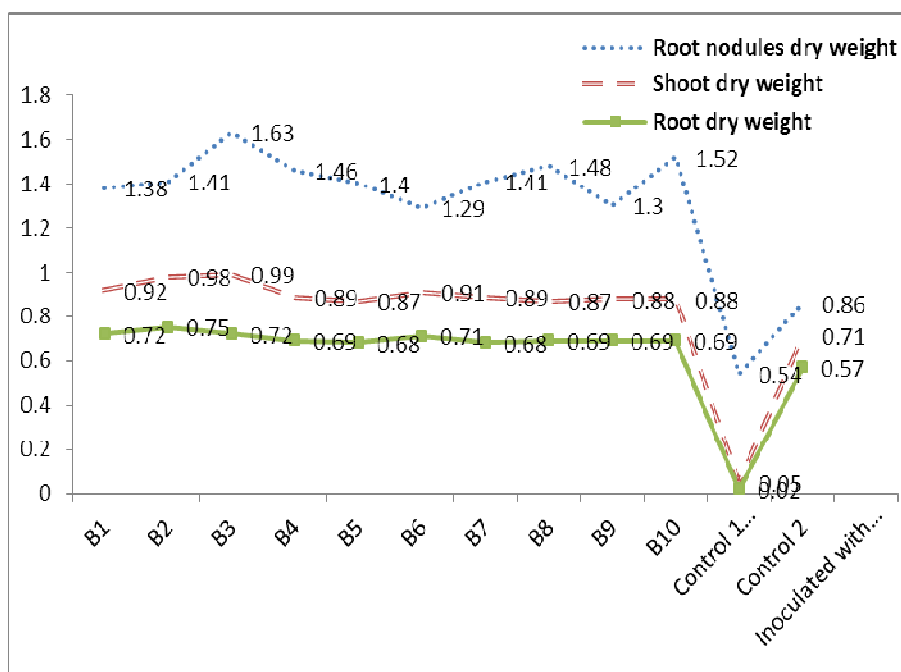


Figure: 3 Dry weight of root nodules, shoot and root of soybean cv. 335 after 32 days of sowing

TABLE: 1 Effect of co-inoculation treatments of *Bradyrhizobium japonicum* (B1) and *Aspergillus niger* Aspnl on number of pods per plant, number of seeds per pod and their dry weight in JS-335 cultivar of soybean

Soybean variety	Treatments	Number of pods/plant	Weight of pods/plant	Number of seeds/pod	Weight of seeds/pod
JS-335	Control	24.5±2.6 ^b	5.5±1.5 ^b	2.75±1.2 ^a	0.6±0.11 ^a
	B1	31.5±3.5 ^a	9.16±1.6 ^a	3±0.6 ^a	0.68±0.12 ^{ab}
	B1 + Aspnl	36.1±5.1 ^{ab}	11.37±1.7 ^{ab}	3.2±1 ^b	0.73±0.18 ^{bc}

No. of pods/plant (F3,6 = 102.153 P<0.001), Weight of pods/plant (F3,6 = 90.754 P<0.001), No. of seeds/pod (F3,6 = 11.986 P<0.01), Weight of seeds/pod (F3,6 = 8.414 P<0.05). The mean ± SE values for three replicates not followed by common lower-case letters are significantly different at P< 0.05 as determined by Duncan's Multiple Range Test

Effect of co-inoculation treatments on pods per plant and dry weight

As evident from Table-1, in cultivar JS-335, B1 isolate of *B. japonicum* produced 31.5 pods/plant, while it was 35.5 in the presence of fungus *A. niger* Aspnl. Also, dry weight of pods per plant was observed to be highest in co-

inoculated pots when compared to controls. A similar trend was observed in all the three treatments with fungal isolate. In case of B3+Aspn1 treated plants pods per plant and their weight showed highest values (Table-3).

Effect of co-inoculation treatments on seeds per pod and dry weight

B2 isolate along with Aspn1 when co-inoculated resulted in higher number of seeds per pod and seed dry weight (Table-2).

In countries including India with rapidly increasing population, soybean is viewed as a crop that enhances nutritive value of the local diets and also shortages of vegetable oil and other dietary supplements. Availability of nitrogen is one important factor that can directly influence plant growth (yield). Biofertilizers increase crop growth by several of mechanisms, including biological nitrogen fixation (BNF), phytohormone production, increasing the availability of soil nutrients and plant disease control [28].

Reports are available that inoculation of *Rhizobium* increase the accumulation of N in the roots thereby resulting in increased root length [29]. Indigenous rhizobial populations fix N₂ with different efficiency depending of their density and activity and affect the root part and nodulation [30], [31]. Similar results were obtained when a field experiment was conducted with soybean to find out the effect of inoculation and scheduling of irrigation on nodulation and nitrogen accumulation in plant parts [24].

TABLE: 2 Effect of co-inoculation treatments of *Bradyrhizobium japonicum* (B1) and *Aspergillus niger* Aspn1 on number of pods per plant, number of seeds per pod and their dry weight in JS-335 cultivar of soybean

Soybean variety	Treatments	Number of pods/plant	Weight of pods/plant	Number of seeds/pod	Weight of seeds/pod
JS-335	B2	29.5±4.5 ^a	8.9±1.8 ^a	3±0.5 ^a	0.61±0.1 ^{ab}
	B2 +Aspn1	32.0±3.6 ^{ab}	10.15±2 ^{ab}	3.5±1.3 ^b	0.75±0.19 ^{bc}
	Control	27.5±3.4 ^b	7.7±0.9 ^b	3.4±0.8 ^a	0.64±0.17 ^a

No. of pods/plant ($F_{3,6} = 102.153$ $P < 0.001$), Weight of pods/plant ($F_{3,6} = 90.754$ $P < 0.001$), No. of seeds/pod ($F_{3,6} = 11.986$ $P < 0.01$), Weight of seeds/pod ($F_{3,6} = 8.414$ $P < 0.05$). The mean \pm SE values for three replicates not followed by common lower-case letters are significantly different at $P < 0.05$ as determined by Duncan's Multiple Range Test.

TABLE: 3 Effect of co-inoculation treatments of *Bradyrhizobium japonicum* (B3) and *Aspergillus niger* Aspn1 on number of pods per plant, number of seeds per pod and their dry weight in JS-335 cultivar of soybean

Soybean variety	Treatments	Number of pods/plant	Weight of pods/plant	Number of seeds/pod	Weight of seeds/pod
JS-335	Control	27.5±3.4 ^b	7.7±0.9 ^b	3.4±0.8 ^a	0.64±0.17 ^a
	B3	34±4.0 ^a	10.4±2.3 ^a	3.25±0.6 ^a	0.64±0.1 ^{ab}
	B3 +Aspn1	38±2.9 ^{ab}	12.3±1.5 ^{ab}	3.1±1.1 ^b	0.69±0.12 ^{bc}

No. of pods/plant ($F_{3,6} = 102.153$ $P < 0.001$), Weight of pods/plant ($F_{3,6} = 90.754$ $P < 0.001$), No. of seeds/pod ($F_{3,6} = 11.986$ $P < 0.01$), Weight of seeds/pod ($F_{3,6} = 8.414$ $P < 0.05$). The mean \pm SE values for three replicates not followed by common lower-case letters are significantly different at $P < 0.05$ as determined by Duncan's Multiple Range Test.

Average number of pods per plant

A significant increase was observed when all three indigenous *B. japonicum* isolates were co-inoculated with *A. niger* (Table-1). PGP microbes have been used in various crops for seed emergence, enhanced growth, and crop yield [22], [27],[32],[33].

Many studies have shown that inoculation with some plant growth-promoting microbes (PGPMs), increases growth and yield in a number of plants especially legumes [34], [35]. Number of pods per plant was significantly higher when the bacterial isolates were applied in combination with fungal isolates compared with the bacterial isolate alone and uninoculated control for soybean cultivar JS-335 in the present study. Similarly, weight of pods/plant, number of seeds/pod, weight of seeds/pod was also statistically significant in relation to inoculation of *B. japonicum* alone. It is reported that certain strains of rhizobia can promote wheat growth and yield through mechanisms that improve single leaf net photosynthetic rate rather than biological N₂ fixation [36].

Average number of seeds/pod

This attribute is the most important aspect because it is directly related to economy of farmers and villagers. The control inoculum showed significant decrease in seeds per pod over co-inoculated treatments with B1 isolate (Table-1). The study revealed that *B. japonicum* could improve the competitive ability and symbiotic effectiveness along with yield index in combination with fungi. Weight of seed is directly proportional to the oil deposition in the seed and high soy protein as well as other nutrients. Positive effect of inoculation by *B. japonicum* was accompanied with increase in seed yield of soybean and our results are more or less in accordance with the findings of earlier researchers [37], [38]. Increase in nodulation and yield of soybean by *B. japonicum* strains have also been reported in other countries like Canada [39] and South Africa [40].

Co-inoculation with rhizobial inoculant and phosphate solubilizing micro-organisms increased seed yield of soybean. It was reported that phosphate solubilizing bacteria individually increased the yield significantly in the pulses [41]. Present study is also positively significant ($p < 0.05$) for seed yield. This means that sustainable agricultural systems will have to intensify production (yield) per unit area. Although, many authors have suggested that the effects of co-inoculation on plants depend on the particular combinations used [42], [43] but in the present study co-inoculation effect of *B. japonicum* and *A. niger* was significantly higher over respective controls. Another interesting observation in the study is that B3 isolate out of three was specific for the test cultivar JS-335, although other two isolates were also found suitable (Table-3) but B3 appeared to be most promising as regards the studied parameters.

CONCLUSION

Use of biological techniques for sustainable crop production is getting popularity throughout the world and the effect of biofertilizers in maintaining the soil fertility has been studied extensively. The use of PGP (Plant Growth Promoting) microbes for reducing chemical inputs in agriculture is a potentially important tool. At this juncture of the study, it appears that coinoculation of more than two growth promoting microbes can supplement each other effects. Secondly results are more promising with coinoculation of native microbes which are well adapted to the edaphic and climatic conditions of the test region. Present study it is the cultivar JS-335 of soybean and Black Cotton Soil that showed a better growth and yield with coinoculation of native *B.japonicum* and *A.niger* strains.

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