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ASSOCIATIVE EFFECT OF *AZOSPIRILLUM BRASILENSE* WITH *RHIZOBIUM JAPONICUM* ON NODULATION AND YIELD OF SOYBEAN (*GLYCINE MAX*)

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Key words

Associative effect *Azospirillum brasilense* Grain yield Nodulation *Rhizobium japonicum* Soybean

Summary

Azospirillum was associated with nodules of soybean. In general, seed inoculation with a broth culture of *Azospirillum brasilense* alone significantly increased nodulation and grain yield of soybean grown in pots in unsterilized soil with different levels of urea ranging from 0 to 80 kg N/ha. This trend was significantly reproducible in a second experiment when a carrier based inoculant of the bacterium was used for seed inoculation.

Inoculation with *Rhizobium japonicum* and *A. brasilense* in combination generally increased grain yield in both the experiments, although the data were not significant.

Introduction

The occurrence of *Azospirillum brasilense* (Syn. *Spirillum lipoferum*) in association with roots of grasses and other economically important plants has been documented[1,2,3,4]. The beneficial effect of Azospirillum inoculation on the yield of crop plants has also been reported[6,7]. However, it is not known whether *A. brasilense* would also colonize roots and nodules of legumes and influence nodulation and yield. Therefore, in the present study, attempts were made to isolate Azospirillum from roots and nodules of some cultivars of soybean. Experiments were also designed to find out the associative effect of known strains of *A. brasilense* with *Rhizobium japonicum* on nodulation and yield of soybean.

Materials and methods

In preliminary studies, six varieties of soybean were grown in unsterilized as well as sterilized soil by sowing seeds inoculated with a known strain of *A. brasilense* (I.A.R.I. strain, Madhu) and *R. japonicum* (I.A.R.I. strain SB-16). Plants grown similarly from uninoculated seeds served as controls. Two months after sowing, plants were carefully up-rooted, washed in running water and root pieces and whole nodules were cultured for Azospirillum following the methods outlined earlier by other workers[1,2,4,5]. For pot trials, farm soil of the following composition from the Indian Agricultural Research Institute was used: moisture 1.2%; total organic carbon, 0.58%; total nitrogen 0.051%; total available P_2O_5, 8.2 ppm; moisture holding capacity, 28–40%; loss on ignition 0.25% and pH 7–8. It was sieved and mixed uniformly with super phosphate at 80 kg P_2O_5/ha and distributed in 14″ diameter pots at the rate of 10 kg soil in each. Different series of pots were maintained having varying quantities of urea from 0 kg N/ha to 80 kg N/ha. Plants were grown in a pot culture house receiving sunlight for 11–12 hours during the day and temperature fluctuated between 26 (min.) to 40°C (maxi). They were watered with tap water uniformly as and when required. *R. japonicum* (I.A.R.I. strain SB-16) and two strains of *A. brasilense* (strain sp-7 from Brazil, courtesy Dr. Dobereiner and I.A.R.I. Madhu strain isolated from roots of rice var. Madhu) were used.

Soybean var. Clark was used as the test plant to evaluate the effects of interaction between the two bacteria. At the end of desired periods, plants were carefully uprooted, washed and data obtained on number and dry weight of nodules and dry weight of shoots. The data on yield of grains or pods were obtained at the time of harvest.

For the first experiment, bacteria were grown on solid media in glass bottles. *R. japonicum* was grown on YEMA (Yeast extract mannitol agar) medium and *A. brasilense* on semi-solid sodium malate medium[1] for seven days. The surface growth of Rhizobium was suspended in sterile water while the semi-solid medium containing Azospirillum was used as such. Seed was inoculated by pouring suspensions of bacteria individually or in combination over seeds (20 ml of rhizobial suspension or 20 ml of Azospirillum suspension or equal aliquots of 10 ml each of Rhizobium and Azospirillum suspension for 100 g of seeds).

By the time the first experiment was completed, it was found that sterilized farm-yard manure-soil (1 : 1) was a useful carrier for Azospirillum in another investigation in the laboratory. Therefore, in the second experiment, the following changes were made: 1) Instead of using bacterial suspension for inoculation, seeds were inoculated with peat-based powder inoculant of *R. japonicum* (20 g/100 g seed) or farm-yard manure-soil based powder inoculant of *A. brasilense* (20 g/100 g seed) or a combination of both the inoculants (10 g + 10 g/100 g seed). In some treatments, autoclaved carrier based *A. brasilense* and *R. japonicum* cultures were used to find out if the carrier itself had any effect.

Results and discussion

The results on isolation of Azospirillum from roots and nodules are presented in Table 1. It was apparent that roots and nodules of soybean had Azospirillum in them which could be isolated by the enrichment method, although in some varieties, the bacterium could not be isolated from uninoculated control series. These results point out that Azospirillum can colonize roots and nodules of *R. japonicum* inoculated soybean grown in unsterilized soil as well as in unsterilized soil inoculated with a known isolate of Azospirillum. There is, however, a need to extend these studies to other leguminous plants.

In both the pot culture experiments, it was obvious that *R. japonicum* inoculation resulted in better nodulation and yield (Tables 2 and 3). Application of urea of 80 kg N/ha without *R. japonicum* inoculation, however, did not result in appreciable increase in grain yield. The noteworthy feature was that seed inoculation with *A. brasilense* alone significantly increased all the parameters of nodulation of roots by native rhizobia at all levels of inorganic nitrogen when I.A.R.I. Madhu strain was used, although dry weight of nodules alone was significantly increased over corresponding controls when the strain Sp. 7 from Brazil was used (Table 2). The increase in the number of nodules by inoculation with *A. brasilense*, Madhu strain alone was however, progressively curtailed with increased levels of urea in soil. Inoculation of Madhu strain of *A. brasilense* alone

Table 1. Recovery of Azospirillum from roots and nodules of different varieties of soybean grown in unsterilized soil with and without seed inoculation of *A. brasilense*

Variety	Inoculated with *Azospirillum brasilense* Strain Madhu (IARI)		Uninoculated control	
	Root	Nodule	Root	Nodule
Ankur	+	–	–	–
Bragg	+	+	+	–
Clark 63	+	+	+	–
Geduld	+	+	+	+
Kalitur	+	+	+	+
Lee	+	+	+	+

+ = present; – = absent.

significantly increased grain yield over corresponding control in the presence of 40 and 80 kg N/ha of urea. In this context, it is relevant to point out that grain yield obtained in *A. brasilense* (Madhu strain) inoculated plants at 40 kg N/ha was equivalent to that obtained in plants receiving 80 kg N/ha without any inoculation. This trend was also seen from the results of the second experiment (Table 3) where a carrier based *A. brasilense* inoculant was used, although not as uniformly as observed in the first experiment.

A combination of the two bacteria significantly increased the number of nodules over corresponding control at 0 kg N/ha, when I.A.R.I. Madhu strain of *A. brasilense* was used (Table 2), while this was not significantly reproducible in the second experiment although slight general increases were apparent (Table 3). However, increase in grain yields over corresponding control was not apparent when both the bacteria were used for seed inoculation in the first as well as the second experiment.

The results in Table 3 clarify the possible influence of the carrier materials used in the preparation of the inoculants. The results also reveal the effects of heat-killed (autoclaved) carrier based inoculants on the yield of soybean at different levels of urea. Generally speaking, the autoclaved carrier alone or the autoclaved carrier based inoculants had no effect on nodulation or yield.

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Table 2. Response of soybean (*Glycine max*) var. Clark 63 to inoculation with *Rhizobium japonicum* and *Azospirillum brasilense* at different levels of urea (Spring 1977) in unsterilized soil – mean of 3 pots, data expressed as per pot

Treatments	Nitrogen (kg/ha) as urea															
	0.00				20.000				40.000				80.000			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Uninoculated control	2.3	0.3	12.7	19.7	7.0	0.4	15.0	21.8	9.3	0.2	14.8	23.0	2.0	0.3	21.2	24.4
R. japonicum (SB-16)	294.0*	2.5*	31.4*	37.7*	307.0*	2.1*	32.6*	37.7*	274.0*	2.3*	31.0*	38.3*	240.3*	1.8*	26.4*	40.8*
A. brasilense Madhu (IARI)	231.3*	2.3*	21.1*	25.9	138.0*	2.1*	28.6*	27.4	126.3*	2.0*	30.0*	32.4*	99.3*	1.7*	31.4*	36.1*
R. japonicum (SB-16) + *A. brasilense*, Madhu (IARI)	386.7*	2.0	30.2	39.0	334.0	2.2*	30.1	42.1	365.0	2.3	34.3	42.6	258.3	2.2*	34.4*	44.5
A. brasilense Sp. 7 (Brazil)	26.3	1.4*	23.6*	22.1	7.0	0.8*	20.9	23.6	10.3	0.7*	21.9	24.2	54.0	0.7*	24.4	25.8
R. japonicum (SB-16) + *A. brasilense*, Sp-7 (Brazil)	26.0	2.3	26.6	34.6	234.3	2.3*	33.3	33.8	250.0	2.3	36.5	37.0	215.7	2.1*	37.2*	40.3
C.D. at 5%	76.1	0.2	5.5	6.6	102.7	0.1	7.3	7.9	107.4	0.1	7.6	4.6	86.3	0.1	4.9	4.8

* Significant at 5% over corresponding control; A = Nodule number; B = Dry weight of nodules (g); C = Dry weight of shoot (g); D = Grain yield (g).

Table 3. Response of soybean (*Glycine max*) var. Clark-63 to seed inoculation with peat-based powder inoculant of *Rhizobium japonicum* and farm yard manure plus soil based powder inoculant of *Azospirillum brasilense* at different levels of urea (Spring 1978) in unsterilized soil – mean of 4 pots, data expressed as per pot

Treatments	Nitrogen (kg/ha) as urea											
	0.00				20.000				40.00			
	A	B	C	D	A	B	C	D	A	B	C	D
Uninoculated control	16.0	0.36	30.4	6.6	15.0	0.19	40.9	3.5	38.0	0.40	40.2	9.5
Carrier alone (autoclaved)	9.3	0.19	23.5	5.7	28.3	0.45	41.4	5.4	16.0	0.54	40.9	5.6
R. japonicum (autoclaved)	12.6	0.34	32.6	5.6	24.6	0.55	43.5	5.1	23.0	0.46	42.3	5.6
A. brasilense (autoclaved)	14.0	0.36	32.1	5.7	19.3	0.30	37.7	5.2	31.6	0.53	41.5	4.5
A. brasilense (live)	48.0	0.95*	42.8	18.9*	74.6*	1.66*	53.8	16.8*	94.6*	1.38*	43.3	16.7*
R. japonicum (live)	129.0*	1.29*	41.8	19.6*	123.6*	1.41*	56.6	16.9*	152.0*	2.15*	51.7*	17.6*
A. brasilense (autoclaved) + *R. japonicum* (autoclaved)	7.3	0.31	27.8	7.5	7.6	0.21	37.7	7.2	10.2	0.11	42.1	4.7
A. brasilense (live) + *R. japonicum* (live)	151.0	1.65	50.1	19.9	158.6	2.11*	58.6	18.4	165.3	2.44	56.1	18.7
C.D. at 5%	35.1	0.41	24.6	2.2	36.5	0.22	17.8	6.6	25.5	0.42	7.1	6.6

* Significant at 5% over corresponding control. A = Nodule number; B = Dry weight of nodules (g); C = Dry weight of shoot (g); D = Grain yield (g).

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