

Effect of *Bradyrhizobium japonicum* on Chlorophyll Content, Nodulation, and Plant Growth in Soybean

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ABSTRACT: Study on effectiveness of *Bradyrhizobium japonicum* on soybean [*Glycine max* (L.) Merr.], local cultivar 'Sathiya' were carried out in the garden soil of Tribhuvan University. Different parameters like nodulation, chlorophyll content in fresh leaves and growth of plant in inoculated and uninoculated plant was studied. Pot experiment was conducted in the green house to evaluate the effectiveness of *B. japonicum* on soybean. It was observed that *B. japonicum* inoculation increased the number of nodules, shoot length of plant and total chlorophyll content in fresh leaves of soybean plant. However, root length was decreased in all inoculated plants.

Keywords: *Bradyrhizobium japonicum*, soybean, symbiosis, chlorophyll, nodule

The nitrogen fixation process between legume and *Rhizobium* referred to as symbiotic relationship. Each organism receives something from the other and gives back something in return. Rhizobia bacteria provide the legume plant with nitrogen in the form of ammonium and legume provides the bacteria with carbohydrates as an energy source. Thus, the formation of effective nodules in soybean [*Glycine max* (L.) Merr.] when inoculated with compatible rhizobia leads to fixation of atmospheric nitrogen making nitrogenous fertilization of the soybean unnecessary. The process is influenced by the interaction of genetic elements in soybean and rhizobia.

Bradyrhizobium japonicum is widely distributed in soil which normally fixes atmospheric nitrogen when they are growing in symbiosis with root of soybean. Curling of root takes place due to secretion of cytokinin, polymixin-B etc. by *Rhizobium* (Dart, 1974) and the host-symbiont specificity is governed by a specific plant protein called lectin which involved in recognition of compatible symbiont. Soybean, being nitrogen fixing legume provides for the production of highly nutritive food in nitrogen deficient soil also. When the seed of soybean is inoculated with *Bradyrhizobium japonicum* increases the nodule number, chlorophyll content and growth of plant (Hoque *et al.*, 1999).

MATERIALS AND METHODS

The soybean seeds local cultivar 'Sathiya' were surface sterilized with 70% ethanol for 1 minute followed by 1% sodium hypochlorite for 4 minutes and finally washed with distilled water for 5 to 6 times. Peat culture of *Bradyrhizobium japonicum* was taken from Nepal Agriculture Research Council (NARC), Khumaltar, Lalitpur. Earthen pots (22 × 24 cm²) were filled with sterilized soil and sand in the ratio of 1 : 1 (at pH 6.5). In one set, each pot with uninoculated seeds were sown and in other sets, each pot with inoculated with *Bradyrhizobium japonicum* seeds were sown. All the pots were kept in green house for 25 days. Continuous care and daily watering (tap water) was provided. Three harvest were made at the interval of 19 days i.e; 32th, 51th and 70th days after seed sowing (DAS). In each harvest four replicates from each group were uprooted and washed then number of nodules per plant, shoot length, and root length per plant were recorded. Total chlorophyll content in fresh leaves of soybean was estimated by the method of (Arnon, 1949) using spectrophotometer (spectronic-21).

The data were analyzed using statistical package for social scientist (SPSS) computer program. Analysis of Variance (ANOVA) in One-Way classification system at significance of variations of means at 5% level was used.

RESULTS

The seedlings inoculated with *Bradyrhizobium japonicum* increased the number of nodules per plant. The number of nodules on 31th, 52th, and 70th DAS was found maximum in inoculated plant. Thus it was found that, the increased in nodule number on 32th, 51th, and 70th DAS by 262.5%, 363.6%, and 301.4% over uninoculated (control) plant (Table 1).

The shoot length of plant was found more in inoculated plant and less in uninoculated plant. Thus it was found that, the increased in shoot length on 32th, 51th, and 70th DAS by 19.8%, 24.8%, and 22.8% over uninoculated plant (Table 1).

The root length of plant was less in inoculated plant over uninoculated plant in all days. Thus it was found that, the decreased in root length on 32th, 51th, and 70th DAS by 22%,

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<Received August 2, 2005>

Table 1. Effect of *B. japonicum* on nodule number, shoot length and root length of soybean.

Days after seed sowing	Nodule number per plant			Shoot length per plant (cm)			Root length per plant (cm)		
	UI	I	% INC	UI	I	%INC	UI	I	%DEC
32	4	14.5	262.5	36.5	43.7	19.8	41.5	32.4	22
51	11	51	363.6	51.4	64.2	24.8	42.3	32.4	23.4
70	14	56.2	301.4	54	67.5	22.8	47.7	43.3	8.6

UI = Uninoculated, I = Inoculated, %INC = Percentage Increased, % DEC = Percentage Decreased

Table 2. Effect of *B. japonicum* on total chlorophyll content in fresh leaves.

Days after seed sowing	Total chlorophyll content in mg/g fresh weight of leaves		
	Uninoculated	Inoculated	% Increased
32	2.24	2.74	22.3
51	2.34	2.80	19.7
70	0.90	1.19	32.2

mg/g = milligram per gram

Table 3. Analysis of variances (ANOVA) of different parameters

Trait	D. F.	Fcal	Ftab.	Significance
Nodule numbers	1, 22	25.197	4.30	0.00
Shoot length	1, 22	5.596	4.30	0.027
Root length	1, 22	6.253	4.30	0.020
Chlorophyll	1, 22	1.557	4.30	0.225

D. F. = Degree of Freedom, Fcal = F value (calculated), Ftab = F value (tabulated)

23.4%, and 8.6% over uninoculated plant (Table 1).

In case of total chlorophyll content in fresh leaves of soybean plant, it was found that, total chlorophyll content was higher in inoculated plant and lower in uninoculated plant. Thus, total chlorophyll content in inoculated plant was increased by 22.3%, 19.7%, and 32.2% over uninoculated plant on 32th, 51th, and 70th DAS respectively (Table 2).

The output of different parameters as revealed by SPSS, ANOVA has been summarized in (Table 3). It was found that, inoculation of *Bradyrhizobium japonicum* had highly significant effect on number of nodules, shoot length, and root length of plant ie; ($P < 0.05$) in all case. However, inoculation of *Bradyrhizobium japonicum* did not show the highly significant effect on total chlorophyll content in fresh leaves ie; ($P > 0.05$) see (Table 3).

DISCUSSION

The present research work has been focused on interaction

between soybean and *Bradyrhizobium japonicum* which helps for better nodulation in plant. However, soil pH, soil texture, soil nutrient, soil temperature, water holding capacity of soil and other physical factors also play the important role for nodulation (Buttery *et al.*, 1998).

It has been found that there is a significant increased in nodule number in inoculated plant than uninoculated plant. It is due to presence of sufficient number of *Bradyrhizobium japonicum*. Thus, inoculated plant had higher nodule number than uninoculated plant that may be due to production of hormones like cytokinin, polymixin-B by bacteria (Dart, 1974) and increased the nodule number. Similarly, Okerake *et al.* (2000) found that nodule number was significantly increased by effective strains of *Bradyrhizobium japonicum*. Thus, higher number of effective nodule has higher dry weight. Solaiman (1999a) reported the higher dry weight of nodules in soybean receiving inoculant and molybdenum (1.5 kg/ha) as compared to uninoculated plant. However, few number of nodules were found in uninoculated plant that may be due to watering, contamination and other physical factors while conducting the experiment. Because pots were kept on field from green house after 25th DAS thus, there is a chance for contamination. Thus, few nodules were found in uninoculated plant.

The shoot length was taken in respective days and found that inoculated plant had higher shoot length than uninoculated plant. That may be due to more nodulation in inoculated plant helps for synthesis of nitrogenous compound essential for plant growth. Similarly, Sekhon *et al.* (1984) found that inoculated soybean plant had higher shoot length than uninoculated plant.

The decreased in root length in inoculated plants may be due to presence of large number of nodules. Larger number of nodules contain larger number of bacteroids, which produce higher amount of Indole-3-Acetic acid (IAA) inhibit cell elongation of root (Noggle & Fritz, 1991).

The total chlorophyll content in fresh leaves of inoculated plant had higher concentration than uninoculated plant. That may be due to the synthesis of nitrogenous compound during nitrogen fixation, which are exported from root nodules in the form of ureides and translocated into leaves which are

used for biosynthesis of chlorophyll (Winkler *et al.*, 1987).

Thus; it can say that, when the plant has higher number of root nodules will assimilates the higher amount of nitrogen in plant body. In this way, better growth of shoot and higher concentration of total chlorophyll in inoculated plant will expected that the better yield over uninoculated soybean plant.

CONCLUSION

From this present research work, it is concluded that the inoculation of *Bradyrhizobium japonicum* in soybean was found effective on nodulation, plant growth and total chlorophyll content in fresh leaves of soybean. However, inoculation of *Bradyrhizobium japonicum* inhibit for root growth due to better nodulation.

ACKNOWLEDGEMENT

The authors are thankful to Central Department of Botany, Tribhuvan University, Kathmandu, Nepal for providing laboratory facilities to conduct this research work. We are thankful to Agronomy Division, Nepal Agriculture Research Council, Lalitpur, Nepal for providing the seeds of soybean.

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