Short Communication

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Evaluation of *Bradyrhizobium japonicum* Strains for Biological Nitrogen Fixation in Soybean

NOORUL HASAN MAHMOOD, AZHAR MASHIATULLAH, HAIDER KHAN AND MUHAMMAD MOHSIN IQBAL
Nuclear Institute for Food and Agriculture, Tarnab, Peshawar, Pakistan

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Soybean is an important leguminous oil crop and a major source of protein. Natural nodulation is poor in the Peshawar valley but artificial inoculation with *Bradyrhizobium japonicum* increases growth and bean yield [1]. The present studies were conducted to see the effect of inoculation with four rhizobial strains on growth, yield and N_2-fixing parameters in Bragg variety of soybean, which is adapted to this location.

The soil used was a silty clay loam with pH 7.9, organic matter 1.2%, total N 0.06%, calcium carbonate 11.2% and clay particles 30.4%. Four strains of B. *japonicum* were used viz; TAL 379, TAL 377, TAL 102 and SL 56. The strains TAL 379, TAL 377 and TAL 102 were obtained from NifTAL, Hawaii, USA, while the strain SL 56 was isolated from soybean nodules from Swat. A control was also maintained making the total number of treatments five. The experiment was laid out according to randomised complete block design. Each treatment was replicated four times. Nitrogen, phosphorus and potash were applied @ 20, 60 and 40 kg/ha in the form of urea, single superphosphate and potassium sulphate, respectively. The experiment was normally irrigated as needed. During the cropping season (June-October), rain fall range was 3.0-43.2 mm, minimum temp. range was 7.7-23.9° and maximum temp. range was 26.8-39.6°C.

Each strain of *Bradyrhizobium japonicum* was grown in yeast extract mannitol broth [2]. Concentrated liquid broth of each *Rhizobium* strain was mixed with sterile peat. This peat-based inoculum was used for artificial inoculation of soybean seeds. Viable rhizobial counts per gram of peat, as determined by plate count method [3], was more than 10^8 cells/g. Seeds were inoculated just before sowing by seed pelleting technique [2]. The seeds were sown manually in the NIFA experimental field. Plot size was 3 x 4 m, row to row distance 30 cm and plant to plant distance was 15 cm.

Five soybean plants were uprooted from each treatment at the flowering (60 days after sowing) and pod initiation (75 days after sowing) stages. Plant and nodule weight (after drying at 70°C for 24 hrs) and average number of nodules per plant were recorded. At the final harvest grain yield was recorded and weight of the plant above ground was taken after air drying the plants for 3 days. For nitrogen analysis, grain samples were dried at 70° for 24 hrs, ground to pass through a 40 mesh screen and analysed for total nitrogen by micro-Kjeldahl method [4]. The data were analysed statistically by the analysis of variance [5]. Duncan multiple range test [6] was employed to test the level of significance among the treatment means.

The effect of inoculation with different rhizobial strains on the growth parameters of soybean cv. Bragg has been shown in Table-1. Largest and significantly highest number of nodules and their dry weights were recorded with the strain TAL 377. Nodule number and dry weights in strain TAL 379 and TAL 102 were not significantly different from each other but significantly higher than the un-inoculated control and those produced by the inoculation with isolate SL 56. The strain SL 56 and the control produced statistically equal number of nodules which were not only fewer in number but whitish in appearance and smaller in size. In the other treatments, nodule size was bigger and their colour was pink. It appears that strain SL 56 could not adapt to the local field conditions because it was isolated from Swat area which is at a higher altitude and where temperature is usually lower than that at NIFA experimental fields. Some of the earlier studies had also shown that soybean crops sown between early April to mid May respond better to artificial inoculation than the crops sown in the hotter months of June and July [7].

Nitrogen concentration in seed gives an indirect measure of nitrogen fixation plus nitrogen taken up from the soil. This was highest in the treatment when inoculated with *Bradyrhizobium japonicum* strain TAL 377. Low nitrogen content of the seed was recorded in plants which were not inoculated and in plants treated with the SL 56.

Biological and grain yields exhibited almost the same pattern as was found for nodulation (Table-1). Maximum biological and grain yields were obtained with the application of NifTAL strain 377. Strain TAL 379 produced equal grain yield but lower biological yield than TAL 377. Biological yield of strain TAL 102 was not significantly different from that of the control or the local strain but grain yield of TAL 102 was significantly higher than these two treatments.

At the flowering stage, a significant positive correlation was observed between nodule number and their dry weights (r=0.913). A significant and positive correlation was also

TABLE 1. EFFECT OF FOUR *BRADYRHIZOBIUM JAPONICUM* STRAINS ON YIELD, NODULATION AND % N IN SOYBEAN AT DIFFERENT GROWTH STAGES.

Treatment	Flowering stage			Pod initiation stage			Yield (kg/ha)		
	Nodule No/ plant	Nodule dry wt g/plant	Plant dry wt g/plant	Nodule No/ plant	Nodule dry wt g/plant	Plant dry wt g/plant	Biological*	Grain	% N in grain
Control	28.2c	0.95c	10.5c	36.5c	1.05c	14.1c	6083d	633c	5.16b
TAL 102	50.2b	1.88b	12.2b	65.7b	1.98b	15.7b	6974c	916b	5.38ab
TAL 377	90.7a	2.31a	13.4a	98.7a	2.47a	17.6a	15707a	1266a	5.55a
TAL 379	52.0b	1.90b	12.0b	60.7b	2.01b	16.6b	8333b	1350a	5.22b
SL 56	31.0c	0.97c	10.9c	39.5c	1.06c	15.0c	6974c	725c	5.25b

Each figure is an average of 4 replicates.Mean values followed by the same letter do not differ significantly at 5% level.* Air dried weight of plant (8-10% moisture).

observed between nodule number and grain yield (r=0.978) indicating that with an increase in nodule number/plant, there was a corresponding increase in grain yield.

The improvement of yield and nitrogen fixing efficiency of soybean can be attributed to the efficiency of *Bradyrhizobium japonicum* strain. Beneficial effects of inoculation with proper strain have been reported by many workers. In this experiment although indigenous *Bradyrhizobium* infected the crop, its ability to nodulate was very poor. Introduced strains outclassed indigenous *Bradyrhizobium.* It has been suggested that the effective strains of *Bradyrhizobium* enjoy a competitive advantage over ineffective strains [8]. From the present study it can be concluded that inoculation with strain TAL 377 can increase yield of soybean under local conditions.

Key words: Nitrogen fixation, Soybean, *Bradyrhizobium japonicum* strains.

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