Pak. J. Bot., 38(3): 851-857, 2006.

RESPONSE OF BLACK GRAM [*VIGNA MUNGO* (L.) HEPPER] TO *BRADYRHIZOBIUM JAPONICUM* INOCULATION UNDER DIFFERENT SOIL AMENDMENT SYSTEMS

ARSHAD JAVAID, RUKHSANA BAJWA AND TEHMINA ANJUM

Department of Mycology and Plant Pathology,
University of the Punjab, Quaid-e-Azam Campus Lahore, Pakistan
e-mail: arshadjpk@yahoo.com

Abstract

Growth, nodulation and yield response of Black gram [*Vigna mungo* (L.) Hepper] to inoculation by two strains of *Bradyrhizobium japonicum* viz., TAL-102 and MN-S was studied in different soil amendment systems. Soil was used either un-amended or amended with farmyard manure (FYM), *Trifolium alexandrianum* L., green manure (GM) or NPK fertilizers. In FYM and GM amendments, inoculation failed to induce any change in nodulation while in un-amended and NPK amended soils inoculation resulted in an increase in number and biomass of nodules. Effect of inoculation was more pronounced in NPK than in un-amended soil. In un-amended and FYM amendment, inoculation failed to induce any significant change in shoot biomass and grain yield while in GM amendment inoculation resulted in a significant reduction in the said parameters. In NPK fertilizers amendment, the enhanced nodulation in response to bradyrhizobial inoculation resulted in a subsequent increase in shoot biomass and grain yield. Grain yield was positively correlated with nodule biomass in FYM and NPK fertilizers amendments.

Introduction

The rhizobia are soil organisms that inhabit the rhizosphere of legumes and other plants. They are a rather more diverse group of organisms than might be supposed but are united by their ability to produce nodules on legumes. These symbiotic N_2-fixing microorganisms are now divided into five genera viz., *Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium* and *Azorhizobium.* The enzyme nitrogenase synthesized in the rhizobia converts atmospheric nitrogen to ammonia which is then assimilated by the host plant. The information enabling rhizobia to fix nitrogen by way of conventional nitrogenase is encoded in *nif* genes which are regulated by a complex set of processes (Dixon, 1987).

Maximum benefits of N_2-fixation by legumes often requires the inclusion of selected strains of rhizobia as seed inoculants especially in soils with low population of these microorganisms. The inoculant strain must be effective in its ability to fix N_2 with the cultivar concerned and possesses the ability to compete for nodulation of the plant with other strains of rhizobia that might be present in the soil. Strain competitiveness is influenced by the genetic diversity of both symbiotic partners (Triplett & Sadowsky, 1992) and the soil environment in which nodulation occurs (Streeter, 1994). Brockwell *et al*., (1995) considered that inoculation is invariably futile in soils with populations greater than 1,000 rhizobia g^{-1} whereas in soils with smaller, less effective populations a response would depend on the ability of the inoculant to compete with rhizobia in the soil. However, there is evidence that a significant response to inoculation is possible in soils containing large numbers of established rhizobia when strains with both superior

N_2-fixation efficiency and nodulation competitiveness are inoculated (Bradley *et al*., 1991; Hungria *et al.*, 1998).

Black gram is an important pulse crop of Pakistan. The present study was designed to assess the relative ability of the two *Bradyrhizobium japonicum* strains viz., TAL-102 and MN-S for nodulation and subsequent effect on crop growth and yield in black gram in different soil amendment systems.

Materials and Methods

Experiment was conducted in earthen pots 20 cm diameter and 30 cm deep. Pots were filled with sandy loam soil having organic matter 1.2%, pH 7.8, nitrogen 0.05%, phosphorus 22 mg/kg and potassium 350 mg/kg. The soil was amended either with farmyard manure (FYM) @ 7 g/100g, *Trifolium alexandrianum* green manure (GM) @ 7 g/100g or NPK fertilizers. A basal dose of 20 mg kg^{-1} N as urea, 30 mg kg^{-1} P_2O_5 as triple supper phosphate and 30 mg kg^{-1} K_2O as potassium sulphate was supplied to the NPK amended pot soil.

Two peat based *B. japonicum* inocula viz., *B. japonicum* st. TAL-102 and *B. japonicum* st. MN-S were obtained from Nuclear Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. *B. japonicum* strain TAL-102 is an exotic strain originally isolated from soybean but is also specific to *Vigna radiata* while *B. japonicum* strain MN-S is a local strain isolated from *V. radiata*. Seeds of *V. mungo* with uniform shape, size and weight were surface sterilized with 3% sodium hypochlorite solution for 10 minutes followed by 4 washings with sterilized water. Seeds were allowed to germinate on moistened filter papers in Petri plates at 25 °C for 24 hours. The partially germinated seeds with emerged radicals were pelted with peat based single strain inocula of *B. japonicum* st. TAL-102 and *B. japonicum* st. MN-S with concentrated sugar solution as an adhesive. Plants were harvested 40 days after sowing (DAS) at 50% flowering stage and 75 DAS at physiological maturity. At each harvest roots were carefully separated from pot soil and thoroughly washed under tap water. Nodules were separated from roots and counted. The fresh weight of nodules was recorded. Root and shoot materials were dried in oven at 75°C to constant weight. At 75 days growth stage data regarding pod number and length, number of seeds per pod, grain yield and harvest index were also recorded. Data were analyzed by applying Duncan's Multiple Range (DMR) Test (Steel & Torrie, 1980).

Results and Discussion

Effect of *B. japonicum* inoculation on nodulation: Nodulation data was collected at flowering stage because at maturity most of the nodules were disintegrated and decomposed. In different soil amendment systems, different nodulation response to *B. japonicum* inoculation was recorded. Nodulation in terms of nodule number and nodule fresh biomass was lower in FYM and GM amended than in un-amended and NPK amended soils (Fig. 1A & B). In FYM and GM amendments, nodulation response of test plant to both the inoculated strains of *B. japonicum* was insignificant. In un-amended and NPK amended soils, both the inoculated strains enhanced number and biomass of nodules. Effect was more pronounced in NPK fertilizer than in un-amended soil (Fig. 1A & B). Earlier workers have reported variable responses of nodulation to rhizobial inoculation in different leguminous crops (Asad *et al.,* 1991; Hafeez *et al*., 2000; Bloem & Law, 2001). Generally it is believed that the success of an inoculant strain is limited by the presence of soil indigenous rhizobial population that competes for nodulation (Asad *et al.,* 1995; Brockwell *et al*., 1995). However, the present study showed that the effectiveness of an inoculated strain depends upon the soil environment like level and source of soil nutrients, and amount and type of soil organic matter. According to Asad *et al*., (1991) low native rhizobial populations are not the only criterion for success with inoculation. In addition the host cultivar, the soil type and environmental factors may also affect the establishment of active N_2-fixing symbiosis. Hungria *et al*., (2001) found that *Sinorhizobium fredii* and other Brazilian fast growing rhizobial strains were less effective under acidic soil conditions (pH 5.1 and 5.4) while their effectiveness was increased when the pH was raised to 6.8 and 7.9.

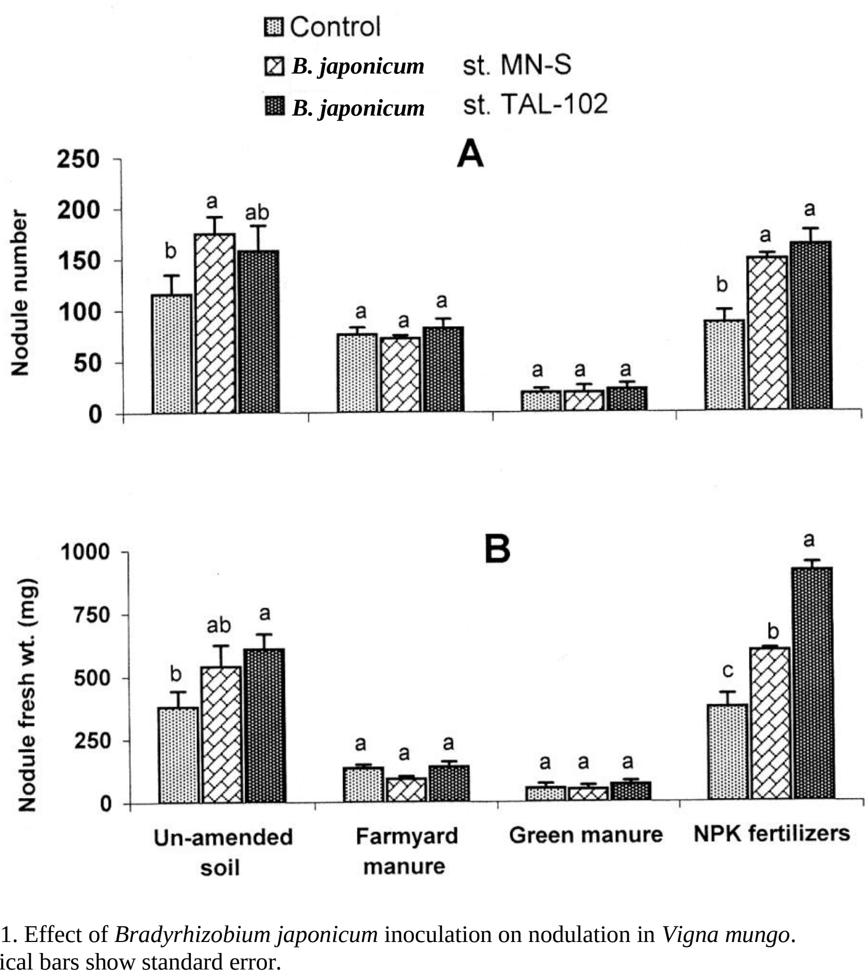

Fig. 1. Effect of *Bradyrhizobium japonicum* inoculation on nodulation in *Vigna mungo*.
Vertical bars show standard error.
In each soil amendment system, values with different letters show significant difference as determined by DMR Test.

Table 1. Effect of *Bradyrhizobium japonicum* inoculation on plant growth in *Vigna mungo*.

Treatments	Flowering stage			Maturity stage		
	Shoot length (cm)	Shoot dry wt. (g)	Root dry wt. (g)	Shoot length (cm)	Shoot dry wt. (g)	Root dry wt. (g)
Un-amended soil						
Control	33 a	3.6 a	0.52 a	31 a	2.9 a	0.53 a
B. japonicum st. MN-S	35 a	4.6 a	0.64 a	38 a	3.6 a	0.60 a
B. japonicum st. TAL-102	35 a	4.1 a	0.46 a	37 a	3.0 a	0.64 a
Farmyar manure						
Control	32 ab	2.2 a	0.14 b	34 b	3.5 a	0.22 a
B. japonicum st. MN-S	30 b	1.7 a	0.16 ab	40 a	3.1 a	0.20 a
B. japonicum st. TAL-102	35 a	2.8 a	0.21 a	38 ab	2.9 a	0.25 a
Green manure						
Control	24 b	1.3 a	0.10 a	29 a	2.3 a	0.19 a
B. japonicum st. MN-S	23 b	2.0 a	0.09 a	25 b	1.3 b	0.09 b
B. japonicum st. TAL-102	29 a	1.5 a	0.10 a	23 b	1.3 b	0.08 b
NPK fertilizers						
Control	34 a	2.8 b	0.34 a	33 a	3.6 b	0.46 b
B. japonicum st. MN-S	33 a	5.4 a	0.35 a	36 a	5.4 a	0.72 a
B. japonicum st. TAL-102	33 a	3.7 b	0.34 a	36 a	4.1 ab	0.69 a

For each soil amendment, values with different letters in a column show significant difference as determined by DMR Test.

Effect of *B. japonicum* inoculation on shoot and root growth: At flowering stage, shoot length and shoot biomass showed an insignificant response to inoculant in all amendment systems except in NPK amendment where a significantly greater shoot biomass was recorded due to MN-S inoculation (Table 1). At maturity, growth response of the test species to inoculation was different than at flowering stage. In un-amended soil, despite increase in nodulation due to inoculation, no significant increase in shoot length and dry matter was observed. Asad *et al*., (1991) have also reported similar nodulation and crop growth response in some grain and fodder legumes to different species of *Rhizobium/Bradyrhizobium* inoculation. This suggests that under some soil conditions, factors other than soil N are the main constraints on plant growth (Owiredu & Danso, 1998; Weiser *et al*., 1990). In FYM shoot length was significantly increased by MN-S inoculation while biomass showed an insignificant response to inoculation of either of the two inoculated strains. Shoot length as well as biomass was significantly reduced by both the inoculated strains in GM amendment. By contrast, MN-S inoculation significantly increased shoot biomass in NPK fertilizer amendments (Table 1).

The root biomass was not significantly affected by inoculation at flowering stage in any amendment system except in FYM where TAL-102 inoculation significantly enhanced the studied parameter (Table 1). At maturity, root biomass in un-amended soil and FYM amended soil were not affected by inoculation while in GM amendment both the strains significantly reduced root biomass. Conversely in NPK fertilizer amendment, inoculation significantly enhanced root biomass (Table 1).

Effect of *B. japonicum* inoculation on yield: Pod number was not affected by inoculation in FYM and NPK amendments. In un-amended soil, there was a significant increase in pod number due to MN-S inoculation. In contrast, inoculation in GM amendment resulted in a significant reduction in pod number (Fig. 2A).

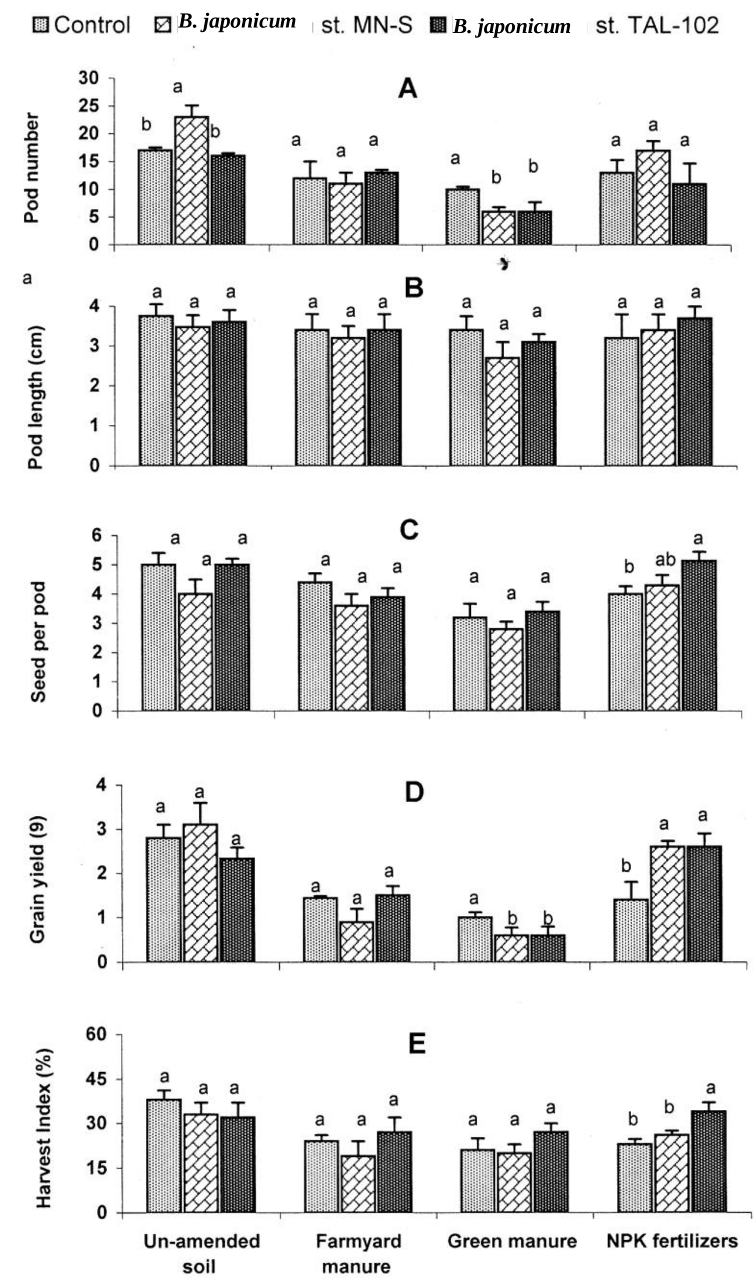

Fig. 2. Effect of *Bradyrhizobium japonicum* inoculation on different yield parameters in *Vigna mungo*.

Vertical bars show standard error.

In each soil amendment system, values with different letters show significant difference as determined by DMR Test.

Table 2. Correlation of nodulation with various plant growth and yield parameters in different soil amendment systems.

	Flowering stage			Maturity stage							
	SL	**SDW**	**RDW**	**SL**	**SDW**	**RDW**	**PN**	**PL**	**SPP**	**GY**	**HI**
	Un-amended soil										
NN	0.96	0.97	0.46	0.99*	0.77	0.79	0.62	-0.99*	-0.72	0.15	-0.90
NFW	0.95*	0.68	-0.11	0.90	0.29	0.99*	0.08	0.08	-0.22	-0.41	-0.99*
	Farmyard manure										
NN	0.99*	0.99*	0.77	-0.21	-0.39	1.0	0.99*	0.80	0.26	0.85	0.96
NFW	0.85	0.85	0.31	-0.70	0.15	0.84	0.90	0.90	0.73	0.99*	0.95
	Green manure										
NN	0.95	-0.21	0.50	-0.71	-0.52	-0.53	-0.50	0.08	0.75	-0.50	0.99*
NFW	0.99*	-0.44	0.69	-0.52	-0.31	-0.31	-0.27	-0.27	0.89	-0.27	0.99*
	NPK fertilizers										
NN	-0.99*	0.64	0.34	0.99*	0.58	0.96	0.02	0.89	0.81	0.99*	0.82
NFW	-0.95	0.25	-0.10	0.86	0.17	0.74	-0.41	-0.41	0.99*	0.81	0.99*

*, **, *** = Significant at 5, 1 and 0.1% level of significance.

SL=Shoot length, SDW=Shoot dry weight, RDW=Root dry weight, PN=Pod number, PL=Pod length, SPP=Seed per pod, GY=Grain yield, HI=Harvest index, NN=Nodules number, NFW=Nodules fresh weight

Pod length failed to show a significant response to inoculation in any of the four soil amendment systems. Similarly number of seeds per pod remained unaffected by inoculation in all amendments except in NPK amendment where TAL-102 inoculation significantly enhanced the studied parameters (Fig. 2B & C).

Similar to that of shoot biomass, the grain yield response to inoculation was insignificant in FYM and un-amended soils while in GM amendment, a significant suppression in the studied parameter was observed. Conversely, in NPK amendment, a significant enhanced grain yield was recorded in response to either of the two *B. japonicum* strains (Fig. 2D). *B. japonicum* st. TAL-102 inoculation significantly enhanced harvest index (HI) in NPK amendment. In rest of the soil amendment systems inoculation failed to alter the HI achieved by the corresponding non-inoculated treatments.

Correlation studies: A variable pattern of correlation between nodulation and crop growth/yield parameters was observed in different soil amendment systems (Table 2). At flowering stage, there was a positive correlation between nodulation and shoot growth parameters in un-amended and FYM amended soils. Similarly shoot length in GM amendment was positively associated with number and biomass of nodules. Conversely, shoot length in NPK amendment was negatively correlated with both the nodulation parameters at flowering stage. At maturity, the shoot length was positively associated with nodulation parameters in un-amended and NPK amendment. All these correlations were either significant (P=0.05) or showed very high correlation coefficient values (Table 2). Pod number, pod length, grain yield and HI were positively correlated with nodulation parameters in FYM amendment. Harvest index in GM and NPK amendment and grain yield in NPK amendment was also positively correlated with nodulation parameters. These correlations were either significant or exhibited very high values of correlation coefficient. HI in un-amended soil was negatively and significantly correlated with nodule number (Table 2).

The present study clearly indicates that the two test *B. japonicum* strains have the potential to increase nodulation with a subsequent increase in crop growth and yield in *V. mungo*. The potential of these strains may be better exploited by using specific doses of NPK fertilizers. Further study is, therefore, needed to optimize the NPK fertilizers dose for better results from rhizobial inoculation program in *V. mungo* and other legumes.

References

Asad, S., K.A. Malik and F.Y. Hafeez. 1991. Competition between inoculated and indigenous *Rhizobium/Bradyrhizobium* spp., strains for nodulation of grain and fodder legumes in Pakistan. *Biol. Fertil. Soil.,* 12: 107-111.

Bloen, J.F. and I.J. Law. 2001. Determination of competitive abilities of *Bradyrhizobium japonicum* strains in soils from soybean production regions in South Africa. *Biol. Fertil. Soil.,* 33: 181-189.

Bradley, R.S., D. Mosquera, M. Asakawa and G. Sanchez. 1991. Promiscuity and responses to rhizobial inoculation of tropical kudzu. *Field Crop Res.,* 27: 267-269.

Brockwell, J., R.J. Roughley and D.F. Herridge, 1995. Population dynamics of *Rhizobium japonicum* strains used to inoculate three successive crops of soybean. *Aust. J. Agric. Res.* 38: 1-74.

Dixon, R. 1987. Genetic regulation of nitrogen fixation. In: *The nitrogen and sulphur cycles*, (Eds.): J.A. Cole and S. Fergusm. Society for General Microbiology Symposium 42, Cambridge University Press.

Hungria, M., L.H. Boddey, M.A. Santos and M.A.T. Vargas. 1998. Nitrogen fixation capacity and nodule occupancy by *Bradyrhizobium japonicum* and *B. elkanii* strains. *Biol. Fertil. Soils,* 27: 393-399.

Hungria, M., R.J. Campo, L.M.O. Chueire, L. Grange and M. Megias. 2001. Symbiotic effectiveness of fast growing rhizobial strains isolated from soybean nodules in Brazil. *Biol. Fertil. Soil.,* 33: 387-394.

Owiredu, J.D. and S.K.A. Danso. 1988. Response of soybean to *Bradyrhizobium japonicum* inoculation in three soils in Ghana. *Soil Biol. Biochem*., 20: 311-314.

Streeter, J.G. 1994. Failure of inoculant rhizobia to overcome the dominance of indigenous strains for nodulae formation. *Can. J. Microbiol.,* 40: 513-522.

Triplett, E.W. and M.J. Sadowsky. 1992. Genetics of competition for nodulation of legumes. *Ann. Rev. Microbiol.,* 46: 399-428.

Weiser, G.C., H.D. Skipper and A.G.H. Wollum.1990. Exclusion of inefficient *Bradyrhizobium japonicum* serogroups by soybean genotypes. *Plant Soil*, 121: 90-105.

(Received for publication 18 October 2004)