SCREENING OF SYMBIOTIC RHIZOBIA ISOLATED FROM ROOT NODULES OF *VIGNA MUNGO* (L.) HEPPER (BLACK GRAM) AND ITS WILD RELATIVES FOR INOCULUM PRODUCTION

D. D. Wasalamuni*, P. Saravana Kumar and E. M. H. G. S. Ekanayake

Department of Botany, Faculty of science, University of Peradeniya

Introduction

The use of rhizobial inoculants as a biofertilizer would enrich soil as *Rhizobium* form nodules in the roots of most leguminous plants and these enable the plants to fix atmospheric nitrogen. Rhizobial inoculants are cost effective, environment friendly and do not promote the growth of weeds (Seneviratne *et al.*, 2000). Black gram (*Vigna mungo*) is one of the most important grain legumes cultivated in Sri Lanka. The extent of cultivation is about 17000ha/year. Urea (a chemical nitrogen fertilizer) application for black gram is about 65 Kg/ha (recommended by Department of Agriculture) which costs nearly Rs 77 million per year. Development of a high quality *Rhizobium* inoculant for black gram is therefore very important. Grain legumes like black gram and green gram (*Vigna radiata*) are promiscuous; nodulate with several species of *Rhizobium* (Somasegaran and Hoben, 1994). Therefore, if a high quality *Rhizobium* inoculant is developed for black gram, it may be possible to use the same inoculant for green gram. The main objective of the present study was to isolate highly infective and highly effective rhizobial isolates from black gram and wild relatives of *Vigna* in order to produce highly efficient inoculants for both black gram and green gram.

Materials and Methods

Root nodules of *Vigna mungo* and wild relatives of Vigna were collected from different locations in Anuradhapura and Puttalam districts of Sri Lanka. Purification and isolation of rhizobia from the collected nodules were done by several successive sub-culturing on half Lupin Agar (LA) medium plates. Eleven isolates were identified. Authentication and screening of high effective strains among them were done as a pot experiment carried out under semi aseptic conditions by using black gram and green gram taken as test plants.

Seedlings (3 days old) of both crops (grown separately in sand based pots) were inoculated with 0.75 ml of bacterial suspension of each isolate separately. Mineral nitrogen fertilizer applied treatment and nitrogen free treatment (neither inoculation no mineral N fertilizer) served as the controls. The treatments were assigned in to Completely Randomized Design (CRD) with three replicates. Plant dry matter production was measured and the number of nodules formed was counted at 6 weeks and 10 weeks after inoculation.

The better performed isolates among the eleven isolates tested were selected and used to test the competitiveness of them with native rhizobia present in soil. This study was conducted under

out-door conditions with pots containing sand and garden soil mixed in 3:1 ratio. Pre-germinated seeds of black gram and green gram were planted in the pots separately. Seedlings were inoculated with 0.75ml of bacterial suspension of the selected isolates separately. Mineral N fertilizer applied and N free treatments served as the controls. The treatments were assigned in to CRD with three replicates. Plant dry matter production and numbers of nodules were taken at 8 weeks after inoculation. The data were analyzed by using SAS software program.

Results and Discussion

Morphologically different eleven types of rhizobia were isolated from *Vigna mungo* and *Vigna* wild relatives. They were numbered from BG1 to BG11 (Figure 1). The results of authentication showed that BG2, BG6, BG7, BG8, BG9, BG10, and BG11 were able to form root nodules after 6 weeks of inoculation. Isolates BG6, BG7, BG8 and BG9 significantly increased the average dry weights of both black gram and green gram plants after 6 weeks and 10 weeks of inoculation than that of the N+ control (Figures 1 and 2). Nodulation with those isolates was better and it enhanced the nitrogen fixation that resulted in higher amounts of dry matter production. These results indicate that isolates BG6, BG7, BG8 and BG9 are the most effective among the evaluated 11 isolates in this study and were selected to test the competitive ability with native rhizobia in the soil. The highest average total dry weight was obtained for black gram plants inoculated with BG8 (Figure 3) and green gram plants inoculated with BG7 (Figure 3). These dry weights were numerically higher than that of the N+ controls and significantly higher than that of the N- controls (Figure 3). These results suggest that isolates BG8 and BG7 can fix nitrogen effectively and support plant growth and are well adapted isolates to compete with native rhizobia found in the soil. Therefore according to the data obtained BG8 and BG7 are the two most suitable isolates to be developed as rhizobial inoculants for black gram and green gram, respectively.

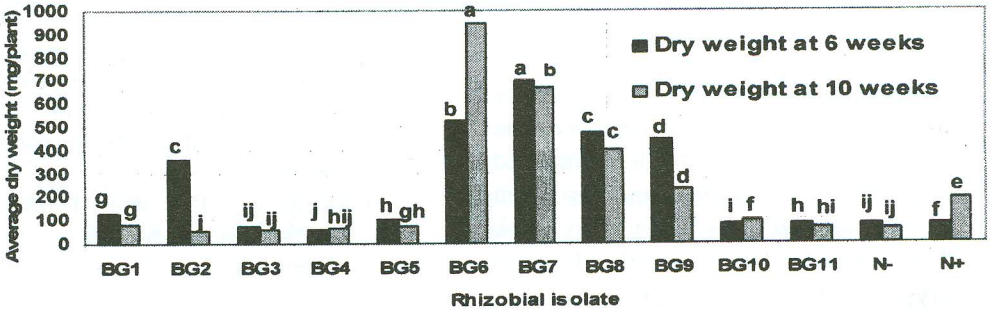

Figure1. Average dry weights of black gram after 6 weeks and 10 weeks of inoculation.

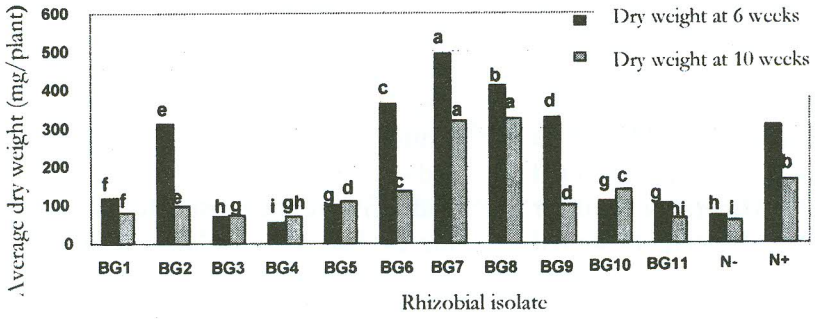

Figure 2. Average dry weights of green gram after 6 weeks and 10 weeks of inoculation.

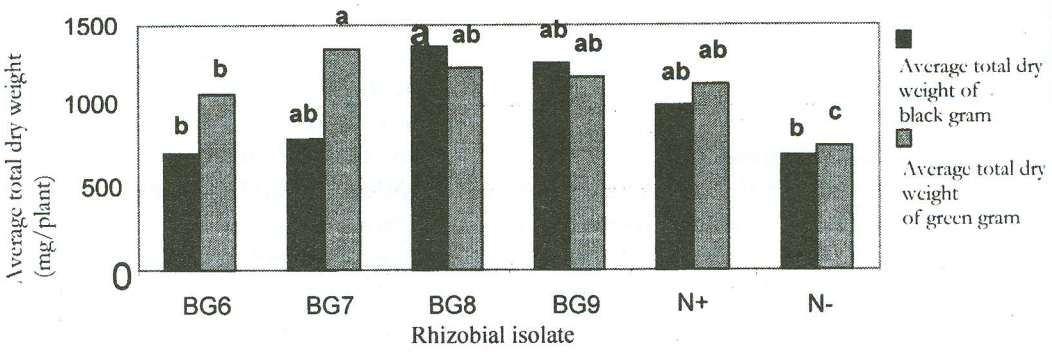

Figure 3. Average total dry weight of black gram and green gram plants after 8 weeks of inoculation.

Conclusions

Among the 11 isolates, BG6, BG7, BG8 and BG9 were the most effective, since they enhanced the nitrogen fixation that resulted in higher amounts of dry matter production in both green gram and black gram plants. Among them BG7 and BG8 were well adapted to compete with native rhizobia found in the soil. For black gram BG8 was the most suitable isolate to be developed as a rhizobial inoculant and for green gram it was BG7. Isolates from black gram increased the growth of green gram plants, therefore cross inoculation is possible.

Acknowledgement

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References

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