SCREENING OF *RHIZOBIUM* ISOLATES ASSOCIATED WITH *VIGNA RADIATA* (L.) Wilczek (GREEN GRAM) TO EXPLORE EFFECTIVE RHIZOBIAL ISOLATES FOR INOCULANT PRODUCTION

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Introduction

The greater success in terms of modified agricultural practices arises from research on legume-rhizobium symbiosis is the development of rhizobial inoculant for the grain legumes (Giller and Cadisch, 1995). In legume crop cultivation, rhizobial inoculants are more sustainable for soil and they are economically beneficial compared to the chemical fertilizers. In Sri Lanka there are no proper inoculants for crop legumes such as green gram and black gram. In our studies in 2008 proved that some *Rhizobium* isolates associated with Green gram were able to nodulate black gram, thus cross inoculation is possible and there is a possibility to use one inoculum to both black gram and green gram (Manamgoda *et al.*, 2008). *Rhizobium* spp. strain selection is a critical step in development of an inoculum (Xavier *et al.*, 2004).

The objectives of this study were to authenticate 12 *Rhizobium* isolates which were used in the previous study, in order to screen effective isolates for green gram and black gram and study their effectiveness with the native *Rhizobium* sp. to explore the potential to use them as inoculants for green gram and black gram.

Materials and Methods

Authentication was done with all 12 isolates (GG1-GG12) under semi aseptic conditions inside a glass house. Two pots were prepared for each isolate by planting green gram and black gram together in one pot. The control pots were treated either with nitrogen or without nitrogen and without any isolate. Inoculation was done after three days of planting seeds. Number of nodules and shoot dry weight were recorded and data were analyzed after 10 weeks. These results together with the results of plants harvested after 6 weeks were used in the screening of effective rhizobial isolates.

Testing the effect of screened isolates with native *Rhizobium* sp. was done as a pot experiment under field conditions. Three seeds each of green gram and black gram were planted in the pots separately. There were six replicates for each treatment per host. There were two nitrogen added control pots and two control pots without nitrogen addition for each host. Approximately 0.75 ml of bacterial cell suspension was inoculated to the root of the plant after a week of planting the seeds. Twenty nine milligrams of urea was added to each pot of Nitrogen added control after 3-4 days after planting. Number of nodules and plant dry weights were taken and data were analyzed after 8 weeks. SAS

computer soft ware was used for analysis.

Results and Discussion

After 10 weeks GG6 isolate performed well with green gram and GG2, GG3, GG4, GG5, GG7, GG8 and GG9 performed well with black gram compared to the nitrogen positive controls (Table 1). Since significantly higher shoot dry weight was given by GG3, GG6, GG8 and GG12 isolates for green gram and GG3, GG7, GG8 and GG12 isolates for black gram after six and ten weeks, they were selected as the high effective isolates in nitrogen fixation for further studies for green gram and black gram respectively. In some cases isolates had performed in a similar manner with both hosts. GG2, GG4, and GG7 isolates with black gram and GG2, GG4, GG6 and GG7 isolates with green gram had increased shoot dry weight and the number of nodules at 10 weeks. These isolates might need more time to establish in the soil and nodulate the host. GG3, GG8 and GG9 isolates showed reduction in their performance and the nodule number after 10 weeks with both hosts. This may be due to nodule decaying and reduction of nitrogen fixing ability. In certain instances even though the number of nodules had increased at 10 weeks shoot dry weight had decreased. This can be observed in GG5 and GG12 isolates with green gram and GG6 and GG12 isolates with black gram. This could be due to excessive uncontrolled nodulation, which would overburden the host plant which can shift the mutualistic balance towards a parasitic relationship.

GG3, GG8 and GG12 isolates with green gram and GG3, GG7 and GG8 isolates with black gram performed well with native *Rhizobium* sp. and showed comparable plant dry weights with the N added control (Table 2). Plants inoculated with the GG3 isolate formed highest number of nodules in both hosts. The well performed isolates had the ability to grow in 0.5%, 1.00%, 1.5%, 2% and 3% salinity conditions and at pH 7 and pH 9. However GG8, GG7 and GG12 were able to grow at pH 5 (Manamgoda *et al.*, 2008). According to Provorov (1984), if antibiotic resistance is associated with improved efficiency, superior *Rhizobium* inoculants can be obtained. GG12 showed resistance for gentamicine, spectomycine and ampicillin. Both GG8 and GG12 were resistant to chloramphenicol, gentamicine, spectomycine, streptomycin and ampicillin. However, GG8 were resistant to tetracycline. GG7 isolate is the most antibiotic tolerant isolate among the 12 isolates, which was also resistant to all above antibiotics and to nalidixic acid & kanamycin (Manamgoda *et al.*, 2008). Therefore these isolates have a greater potential to be used as rhizobial inoculants with various soil conditions.

Conclusion

Rhizobium isolates GG3, GG8 and GG12 have the potential to be used as rhizobial inoculants for green gram. *Rhizobium* isolates GG3, GG8 and GG7 have the potential to be used as inoculants for black gram. Therefore either GG3 or GG8 have the possibility to be used as *Rhizobium*

inocula for both black gram and green gram.

Acknowledgement

Financial assistance from University Research Grant RG/2007/41/S is acknowledged.

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Table 1. Average Shoot Dry Weight (ADW) per plant, Average Number of Nodules (ANN) per plant after 6 weeks and 10 weeks in authentication and their Dunken Grouping (DG).

Isolates	Green gram				Black gram			
	6AD	10ADW	6ANN	10ANN	6ADW	10ADW	6ANN	10AN
GG1	98 ^{GH}	131 ^{ED}	0	0	127 ^{I}	112 ^{I}	0	1
GG2	130 ^{F}	200 ^{C}	2	10	191 ^{G}	477 ^{E}	0	30
GG3	295 ^{A}	204 ^{C}	12	7	627 ^{A}	640 ^{C}	34	31
GG4	168 ^{CD}	238 ^{B}	3	8	258 ^{F}	569 ^{D}	13	41
GG5	171 ^{CD}	156 ^{D}	3	4	195 ^{G}	568 ^{D}	8	20
GG6	155 ^{E}	278 ^{A}	1	6	363 ^{D}	213 ^{G}	18	21
GG7	117 ^{GF}	217 ^{CB}	3	7	313 ^{E}	970 ^{A}	15	41
GG8	186 ^{C}	145 ^{D}	2	0	396 ^{C}	933 ^{B}	28	24
GG9	121 ^{F}	101 ^{GF}	0	1	175 ^{H}	411 ^{F}	7	65
GG10	131 ^{F}	108 ^{EF}	0	0	199 ^{G}	135 ^{I}	6	0
GG11	182 ^{CD}	77 ^{GH}	1	0	192 ^{G}	181 ^{H}	16	3.5
GG12	239 ^{B}	104 ^{EF}	7	11	604 ^{B}	106 ^{I}	26	30
N+	87 ^{H}	72 ^{H}	0	0	134 ^{J}	207 ^{GH}	0	0
N-	165 ^{DE}	267 ^{A}	0	0	61 ^{I}	57 ^{J}	0	0

6 ADW- Average shoot dry weight per plant after 6 weeks in milligrams, 10 ADW- Average shoot dry weight per plant after 10 weeks in milligrams, 6 ANN-Average number of nodules per plant after 6 weeks, 10ANN- Average number of nodules per plant after 10 weeks. The dunken grouping is shown on the top right hand of the shoot dry weight

Table 2. Effectiveness of the screened rhizobial isolates with the native *Rhizobium* sp.

		GG3	GG8	GG12	GG6	GG7	N+	N-
Green gram	ADW	1248.6 ^{A}	1287.2	1055.9	719	-	1171.7	759.7
	ANN &	13 ^{A}	8 ^{A}	3 ^{C}	3 ^{C}	-	7 ^{B}	1 ^{C}
Black gram	ADW	1071 ^{AB}	1675.2	729.5 ^{B}	-	936.6	1000	700.3
	ANN &	12 ^{A}	6 ^{B}	8 $^{A B}$	-	6.6 ^{AB}	4.6 ^{B}	6.6 ^{B}

ADW- Average total plant Dry Weight per plant in milligrams DG- Dunken Grouping ANN- average Number of Nodules per plant