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Response of *Glycine max* to Inoculation with Rhizobial Strains Isolated from Crop Wild Relatives of *Vigna* spp., *Crotalaria* spp. and *Mimosa* spp.

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Abstract: Inoculation of grain legumes with rhizobia has been recorded to have a great potential in maximizing biological nitrogen fixation in these crops. *Glycine max* (soybean) is one of the most important grain legumes in the rain fed farming systems in dry and intermediate zones of Sri Lanka. Rhizobia of wild non-edible legumes have higher tolerance to prevailing adverse conditions. The main objective of the current study was to test the ability of certain rhizobial isolates from wild legumes, which could overcome competition by indigenous strains and effectively nodulate *G. max* in order to use them as rhizobial inoculants. Rhizobial strains from wild legumes (C10, M5, VD1, and VW2) which were previously selected as effective were used in all experiments. Physiological and biochemical characterization of rhizobia was done by assessing their growth in media with different pHs (5, 7 and 9), salt concentrations (0.5 %, 1 %, and 2 %) and sugars (Lactose, Sucrose and Maltose). A field test was conducted with the above four strains, using a Randomized Complete Block Design (RCBD) with three replicate blocks per treatment. Seeds were mixed with coir based inoculants prior to sowing. Samples of nine plants were collected from each treatment after 8 weeks (at early pod filling) for nodules. These plants were oven dried and weighed. Yield and yield component data were recorded in the remaining plants. Strains C10, M5 and VW2 grew at basic pHs. All the strains showed moderate tolerance to salinity except strain M5 which showed a high tolerance to 0.5% and 1% NaCl. Strain VW2 showed a higher ability to use all carbohydrates while strain M5 showed moderate ability. Inoculation with all four strains showed increases in nodulation compared to the uninoculated N+ and N- controls. Dry matter production with strains C10, VD1 and VW2 were significantly higher which was similar to the N fertilizer application. The highest Average Number of Pods was observed with strain VD1. Strain C10 gave the highest value for number of seeds per pod which is significantly higher than the other treatments. With respect to 100 seed weight strains C10 and VD1 gave significantly higher values than the uninoculated controls. All the inoculated treatments gave higher values for seed yield than the uninoculated treatments whereas strains C10 and VD1 gave significantly higher values. Strains C10, VD1 and VW2 appear to be capable of overcoming competition by indigenous rhizobia and suitable to be used as inoculants for *G. max.*

Keywords: Biological nitrogen fixation, *Crotalaria* spp., *Glycine max*, *Mimosa* spp., *Rhizobium*, Rhizobial inoculants.

I. INTRODUCTION

The greatest success in terms of modified agricultural practices arising from research on legume-*Rhizobium*symbiosis has been the development of rhizobial inoculants for grain legumes. Inoculation of grain legumes with rhizobia has been recorded to have a great potential in maximizing biological nitrogen fixation in these crops. Inoculation has the potential of increasing dry matter yield, N yield, and residual N levels [2]. In the production of inoculants rhizobia have been isolated, characterized and selected for infectivity and effectiveness. Such efficient strains have been embedded in carrier material (peat, rock phosphate, coir dust, etc.) to prepare inoculants which are used to inoculate targeted crops to form effective N_2- fixing root nodules. Use of inoculants could reduce the application of chemical Nitrogenfertilizer to these crops thereby minimizing cost of crop production and environmental pollution, particularly important in developing countries. When considering natural rhizobia of wild non-edible legumes, they have higher tolerance to prevailing adverse conditions such as salt stress, elevated temperatures and drought [1]. Soybean (*Glycine max*L.) is the mostimportant grain legume crop in the world in terms oftotal production and international trade. Soybean seeds contain 18% to 23% oil and about 38% to 44% protein [6]. Soybeanis one of the most important grain legumes in the rain fed farming systems in dry and intermediate zones of Sri Lanka. The main objective of the current study was to test the ability of certain rhizobial isolates from wild legumes, which could overcome competition by indigenous strains and effectively nodulatesoybean in order to use them as rhizobial inoculants.

II. Materials and Methods

The four strains C10 (from *Crotalariabrownei*), M5 (from *Mimosapudica*) VD1 and VW2 (from *Vigna* wild relatives) were selected based upon the results of an earlier pot experiment under aseptic conditions where they were screened for high infectivity and effectiveness [5].

Table 1: The rhizobial strains used

Host species	Location	Code
Crotalariabrownei	Anamaduwa	C10
Mimosa pudica	Kekirawa	M5
Vignadalzelliana	Nawalapitiya	VD1
Vignatrinervia	Kithulhitiyawa	VW2

Physiological and biochemical characterization: To determine the pH tolerance of each rhizobial strain, they were cultured on growth in media at pH 5 (Sodium acetate), 7 (MOPES) and 9 (Tris–HCl). Plates containing, 0.5%, 1% and 2% NaCl, determined the salt tolerance of each strain. Thecarbohydrate utilization of the strains was tested using Lactose, Sucrose and Maltose. Rhizobial cultures were prepared as follows. A loopful of bacteria was taken from a full grown culture on a Petri plate and inoculated into a 5 ml of broth culture. The broth culture was incubated on an orbital shaker at room temperature. After 3 days 1 ml of the broth culture was transferred to a sterilized Eppendorf tube and centrifuged at 3000 rpm for 3 minutes. The supernatant was discarded and the pellet was re-suspended in 0.89% NaCl (saline) solution and centrifuged at 3000 rpm for 3 minutes and the supernatant was discarded. This procedure was repeated and a 10^{-1} dilution suspension was prepared for inoculation on ½ LA (Lupine Agar) medium.

Field test: A field test was conducted at the Department of Botany, Faculty of Science, University of Peradeniya, Sri Lanka. A Randomized Complete Block Design (RCBD) was used with three replicate blocks per treatment. The plot size was 2 m X 1m. Distance between plants x rows was 5cm×40cm. Nitrogen positive and negatives were used as controls. Rhizobial inoculants were prepared by injecting broth cultures of individual rhizobia into autoclaved, powdered and packeted coir dust. The number of rhizobial cells of the inoculum was ×109. Seeds were mixed with the coir based inoculum and sown [7]. Plots were irrigated once a week and recommended agronomic practices were applied. A basal dressing of fertilizer Urea 50 kg/ha (only for the N+ control), TSP

150 kg/ha, MOP 75kg/ha (to all the treatment) was added 12 days after sowing seeds. A top dressing of 50 Kg/ha of urea was added at flowering stage, only to the nitrogen positive controls. Samples of nine plants were collected from each treatment after 8 weeks (at early pod filling).Before harvesting, the plants were visually rated according to their growth performances (scale 1-9). The number of nodules, nodule size, nodule colour and the attachment of the nodules were recorded. These plants and nodules were oven dried at 70°C for 48 hours and weighed. Yield and yield components data were recorded after harvest of mature seeds in the remaining plants. The data were analyzed statistically using ANOVA and DMRT (SAS 1997).

III. Results and Discussion

With respect to carbohydrate utilization strain VW2 showed the highest ability for all the sugars tested and strain M5 showing moderate ability (Table 2). Strain VW2 possess the highest ability to survivewith wide range of carbohydrates. Rhizibial strain differences have been reported in previous studies with regard to different pHs of the culture medium [4]. The strain VD1 is moderately tolerant to all the tested pHs. Therefore, this strain can moderately survive both acidic and basic soils. Strain M5 prefers neutral and basic pHs while strains C10 and VW2 prefer basic pHs. Salinity, a composite stress having both an ionic as well as osmotic component, can be extremely detrimental for the growth of soil-inhabiting bacteria like *Rhizobium* [8]. All the strains were moderately tolerant to tested ranges of salinity except strain M5 which showed higher tolerance to 0.5% and 1% salinity. According to the Physiological and biochemical characterization results, most of the strains have the ability to survive under tested conditions. Therefore, these strains have the capability of surviving under stress conditions.

Table 2: Carbohydrate utilization, pH and salinity tolerance of the rhizobial strains

Strain	Carbohydrate utilization			pH			Salinity(NaCl)		
	MA	LA	SU	5	7	9	0.5%	1%	2%
C10	M	M	L	L	M	H	M	M	M
M5	M	M	M	L	H	H	H	H	M
VD1	L	M	M	M	M	M	M	M	M
VW2	H	H	H	M	M	H	M	M	M

L= Low M= Moderate H= High

The four rhizobial strains tested gave promising results for infectivity and effectiveness in a previous pot experiment in sterilized soil under aseptic conditions. The field testing of the rhizobial strains was conducted primarily to assess their ability to overcome competition from the indigenous rhizobia present in the unsterile field and to observe successful nodulation with this host plant [2].

Results presented in Table 3 show differences among the treatments with respect to Average Visual Rate (AVR). The highest AVR was found with strain C10 whereas lowest value was found in N-control. All the strain inoculated treatments gave higher values than the N- control meaning that the rhizobial inoculated treatments show a good response. All the plants that were inoculated had significant increases in the Average Number of Nodules (ANN) when compared to the uninoculated controls. Highest Average Number of Nodules as well as highest Average Nodule Dry Weight (ANDW) was observed with strain C10 treatment. Addition of mineral fertilizer had a negative effect on nodule formation; hence, there were no nodules in the N+ control. When considering the Average Total Dry Weight (ATDW) strains C10, VD1 and VW2 gave significantly higher values which are similar to N+ control.

The highest Average Number of Pods (ANP) was observed with strain VD1 whereas the least was in the N- control (Table 3). All the rhizobial inoculated treatments gave significantly highervalues than the N+ control with respect to ANP. Although the strain C10 gave the highest value for the APL, it is not significantly higher when compared to the other treatments. Strain C10 gave the highest value for number of seeds per pod which is significantly higher than the other treatments. With respect to 100 seed weight strains C10 and VD1 gave significantly higher values than the N+ and N- controls. When considering the seed yield all the inoculated treatments gave higher values than the N+ and N- controls whereas strains C10 and VD1 gave significantly higher values. Similar results were obtained by Brar and Lal [3] who also found an increase in seed yield with rhizobial inoculation. These results show that rhizobial inoculatnshave capability of replacing chemical Nitrogen fertilizer.

Table 3: Plants Visual Rate, Nodule Number, Nodule Dry Weight and Total Dry Weight in *G. max*

Strain	**AVR**	**ANN**	**ANDW**	**ATDW**
C10	9.00	1.6317[a]	0.0678[a]	5.5550[a]
M5	8.00	1.5851[a]	0.0432[ab]	3.3691[b]
VD1	8.60	1.6071[a]	0.0542[ab]	5.0178[a]
VW2	8.20	0.5655[b]	0.0346[ab]	5.0370[a]
N-	6.00	0.0602[c]	0.0048[b]	1.7295[c]
N+	8.88	0.0000[c]	0.0000[b]	4.9543[a]

AVR=Average visual rate ANN =Average no. nodules/plant (log10) ANDW=Average Nodule dry weight (g/plant) (log10) ATDW=Average Total Dry Weight (g/plant)[a] Mean of five replicates. Values in the same column followed by the same letter are not significantly different at 5% probability level.

IV. Conclusion

The strain C10 appears to be the most suitable as rhizobial inoculants for *G. max*. StrainsVD1 and VW2 can also be used as rhizobial inoculants to overcome competition from indigenous rhizobia and improve biomass production and yield of the host plants.

Table 3: Yield and yield components in *G. max*

Strain	ANP	APL	No seeds/pod	100 seed weight	Seed yield (kg/ha)
C10	32.66[a]	3.01[a]	2.75[a]	10.5292[a]	2369.07[a]
M5	27.20[ab]	2.94[a]	2.13[b]	9.6992[ab]	2182.32[ab]
VD1	33.00[a]	2.88[a]	2.05[b]	10.9652[a]	2467.17[a]
VW2	28.40[ab]	2.91[a]	2.00[b]	9.665[ab]	2174.63[ab]
N+	25.00[b]	2.84[a]	1.94[b]	8.6493[bc]	1946.09[b]
N-	22.00[c]	2.50[ab]	1.86[b]	7.6996[bc]	1732.41[c]

ANP=Average Number of Pods, APL=Average Pod Length.

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