SCREENING OF INDIGENOUS RHIZOBIA ISOLATED FROM ROOT NODULE OF *CROTALARIA* AND *MIMOSA* FOR THEIR INFECTIVITY AND EFFECTIVENESS

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Introduction

Application of chemical Nitrogen fertilizer has today become a common practice in agriculture. Less than 40% of the applied Nitrogen fertilizer is absorbed by crops and the rest is lost except for a small part that is temporarily immobilized in soil (Kulasooriya *et al.*, 2004). Nitrogen lost in this manner is carried away with water and resulting in the pollution of water bodies. In addition, chemical fertilizers cause decrease in organic carbon, reduction in soil microbial flora and increase soil acidity (Nandasena, 2004). Many legumes have the ability to form nitrogen fixing root nodules with rhizobia and thus contribute to the biological fixation of Nitrogen. 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). Rhizobial inoculants are environmental friendly, low in cost, and could be substituted for chemical N fertilizer. Identification of highly infective and effective rhizobia is very important in inoculum production.

The objective of this study was to evaluate Rhizobial strains from two indigenous legumes species *Crotalaria* and *Mimosa* to use as a potential source of inoculant production.

Materials and Methods

Collection of root nodules and isolation of rhizobia

Root nodules were collected from *Crotalaria* and *Mimosa* grown in Anamaduwa, Bulagala, Kalladiya, Kekirawa, Madurankuliya, Mahailluppallama, Naula, Paduwasnuwara, Palapathwala and Puttalum in Sri Lanka. Nodules were surface sterilized, crushed and rhizobia isolated using Congo Red Yeast Mannitol Agar (CRYMA) medium (Somasegeran and Hoben 1994). Pure cultures of rhizobia were stored at -18^{0}C with 20% Glycerol. Ten isolates were obtained from *Crotalaria* sp. and twelve isolates were obtained from *Mimosa* sp.

Authentication and screening of the isolated rhizobia

Authentication and screening of the isolated *rhizobia* was carried as a pot experiment under semi-aseptic conditions. Seedlings of the two host species grown in nitrogen free sterilized sand base potting medium were inoculated with 1 ml of broth inoculum. Three replicates of each isolate were used, with Nitrogen positive and Nitrogen negative controls and they were arranged in to

Complete Randomized Design (CRD). Visual rating (scale of 0-10) based on plant growth performance was done at 6 and 10 weeks after planting. At the same time plant number of nodules were recorded. Data was analyzed using the SAS computer soft ware.

Physiological and biochemical characterization of rhizobia

This was done by observing the growing ability of isolates in media containing different pH, antibiotic and salt conditions. pH 5, 7 and 9 were selected to determine the pH tolerance. Intrinsic antibiotic resistance of the isolates was determined by observing the growing ability of isolates in Ampicillin (AMP), Chloramphenicol (CM), Gentamycin (GM), Kanamycin (KM). Nalidixic acid (NX), Spectonomycin (SP) and Streptomycin (STR). 0.5%, 1%, 1.5%, 2% and 3% NaCl conditions used to determine the salt tolerance.

Results and Discussion

Nodules of *Crotalaria* sp. were light pink in colour and fan shaped while those of *Mimosa* sp. were light pinkish brown and finger shaped. Highest visual rates were observed with mineral N application in both 6 and 10 week old plants. C1, C2, C8 and C10 showed comparatively higher visual rates among the isolates tested. This could be attributed to nodule formation (Figure 1). Isolate C2 showed highest number of nodules after 10 weeks of growth (Average 24.25).

Among the *Mimosa* Rhizobial isolates M4, M5, and M12 showed a higher nodule number per plant after 6 weeks of growth whereas M2, M3, M5 and M9 showed a higher nodule number per plant after 10 weeks of growth (Figure 2). However, M5 and M6 showed higher visual rates as well as higher number of nodules per plant in both the 6 and 10 weeks old plants. This indicates that these two strains have higher infectivity as well as higher effectivity in Nitrogen fixation. A positive correlation was observed between the visual rating and number of nodules per plant in the plants inoculated with strains M3, M4, M5, M6 and M12. When considering the characterization results of *Crotalaria* sp. isolates they were highly tolerant to pH 7 and pH 9 and moderately tolerant to pH 5. They were sensitive to at least one antibiotic. C4, C5 and C9 were highly tolerant to AMP. C4 was highly tolerant to CM and NX. For SP, C5 and C7 were highly tolerant. In this study, antibiotic resistance of the isolates was tested as the basis for selecting a diverse subset of bacteria for further characterization. According to the salinity tolerance results of all isolates showed moderate or low tolerance to all the tested salinity conditions except isolate C7 which was highly tolerant (0.5% NaCl). Therefore, C7 could be developed as a inoculant for saline tolerant crop legumes.

pH tolerance of *Mimosa* sp. isolates were parallel to those of *Crotalaria* sp. isolates. All the *Mimosa* sp. isolates were resistant to AMP and SP. Only M6 was sensitive to CM and only M12 was sensitive to TC. With respect to salinity tolerance results, M5, M6, M7 and M8 were highly tolerant to both 0.5% and 1% salinity conditions.

Conclusions

Based on the experimental data obtained it could be concluded that rhizobial strain C1, C2, C8 and C10 of the *Crotalaria* and M3, M4, M5, M6 and M12 of *Mimosa* showed the best performance and could be recommended for cross inoculation studies with edible legumes.

Acknowledgement

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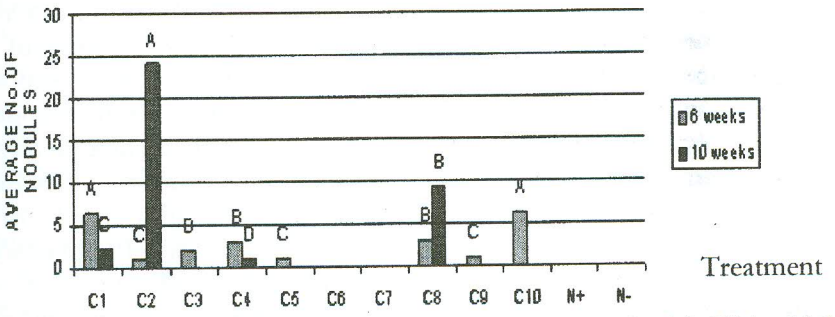

Figure 1. Average no. of nodules of *Crotalaria* sp. inoculated with Rhizobial isolate C1-C10 and in N+ and N- treatments after 6 and 10 weeks of growth. Dunken grouping is shown on the top of the bar. CV%= 18.9.

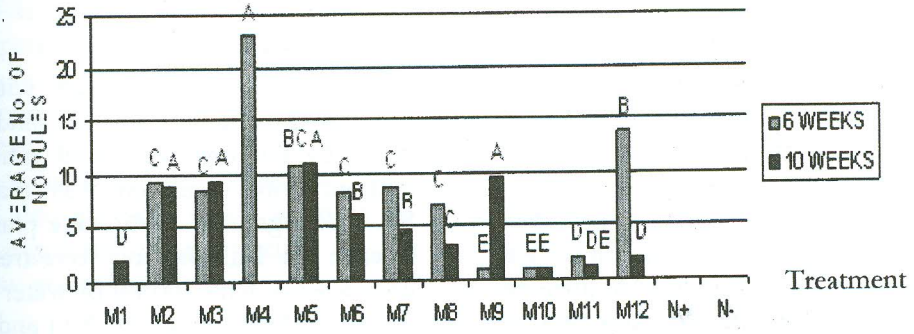

Figure 2. Average no. of nodules of *Mimosa* sp. inoculated with Rhizobial isolate M1-M12 and in N+ and N- treatments after 6 and 10 weeks of growth. Dunken grouping is shown on the top of the bar. CV%= 15.38.