A STUDY ON GENETIC RELATEDNESS OF *RHIZOBIUM* SPECIES NODULATING LEGUME CROPS IN SRI LANKA

M. S. K. Amarakoon and R. P. Karunagoda

Department of Agricultural Biology, University of Peradeniya.

Introduction

Sri Lanka is an agricultural country which has a high tendency in using inorganic fertilizers. Apparently, there are many disadvantages in using inorganic fertilizers such as increasing soil acidity and disrupting the soil structure. The relationship between *Rhizobium* and its legume host ensures the compensation of nitrogen, which is removed with crop harvest. Further, biological nitrogen fixation reduces the above mentioned environmental hazards caused by inorganic fertilizers and the cost encountered with the inorganic fertilizers.

It is a prerequisite that locally available rhizobia be characterized in order to use them beneficially. There have been many conventional techniques, based on morphology and physico-chemical methods to characterize rhizobia. However, they may not give accurate information for identifying the strains as provided by molecular markers. The objective of this study was to characterize different strains of *Rhizobium* associated with legume crops cultivated in Sri Lanka using Randomly Amplified Polymorphic DNAs (RAPDs) to identify their genetic relatedness.

Materials and Methods

Rhizobium strains were isolated from legume crops such as black gram (*Vigna mungo*), seed cowpea (*V. unguiculata var unguiculata*), mae (yard long bean, *V. sesquipedalis*), mung bean (*V. radiata*), soybean (*Glycine max*) and vegetable cowpea (*V. unguiculata var unguiculata (Bushita)*), which were collected from the Field Crops Research and Development Institute at Mahailluppallama, Sri Lanka. Rhizobia were isolated from surface sterilized nodules and streaked on Congo Red Yeast Manitol Agar (CRYMA) plates and incubated at 26°C. One isolate from each crop was used for the study. Total DNA from each strain was extracted according to method described by Karunagoda (1994). The DNA were quantified by measuring the absorbance value at 260 nm. The RAPD analysis was carried out using OPN 06, OPN 07, OPN 09, OPN 11, OPN 14, OPN 20, OPP 01 and OPP 20 random primers (3 min at 94°C, 3 min at 35°C for annealing and 2 min at 72°C for extension; with 35 cycles). The extraction mixture for PCR amplification was contained 1.5 µl of 10 × PCR buffer, 1.5 µl of 25 mM $MgCl_2$, 1.2 µl of 10 mM dNTP mixture, 1.0 µl of 10 pM primer, 0.18 µl of Taq DNA polymerase and 5.0 µl of 25 ng/ µl template DNA in a total volume of 15 µl. The PCR product was run on a 1.5% agarose gel, stained with ethedium bromide and observed under UV illumination.

Results

The colonies which were pink in colour on CRYMA plates were confirmed as *Rhizobium*. From the RAPD analysis, only OPN 06, OPN 07, OPN 09, OPN 14, OPN 20 and OPP 01 produced reproducible bands. The primer OPN 06 did not produce any bands with the strain from vegetable cowpea. Black gram and cowpea isolates produced one monomorphic band, whereas all the others were polymorphic. With the primer OPN 07, one clear polymorphic band was produced by the isolates from cowpea, vegetable cowpea and green gram. The primer OPN 14 did not produce any clear bands with green gram and black gram isolates, whereas, isolates from cowpea, vegetable cowpea and soybean produced monomorphic bands. The primer OPP 01 gave clear banding patterns with all the isolates, having a single monomorphic band sharing with vegetable cowpea, yard long bean and soybean isolates and another monomorphic band sharing with seed cowpea, vegetable cowpea and soybean isolates. The same primer produced a monomorphic band with yard long bean and soybean, too. Monomorphic bands were observed in cowpea, green gram and black gram isolates, and in yard long bean and soybean with the primer OPN 09. The OPN 20 primer produced one monomorphic band with cowpea, vegetable cowpea, yard long bean and black gram isolates, and another monomorphic band between cowpea, green gram, black gram and soybean isolates. Based on the above banding pattern, the following dendogram was developed using POPGENE computer software package (Figure 1).

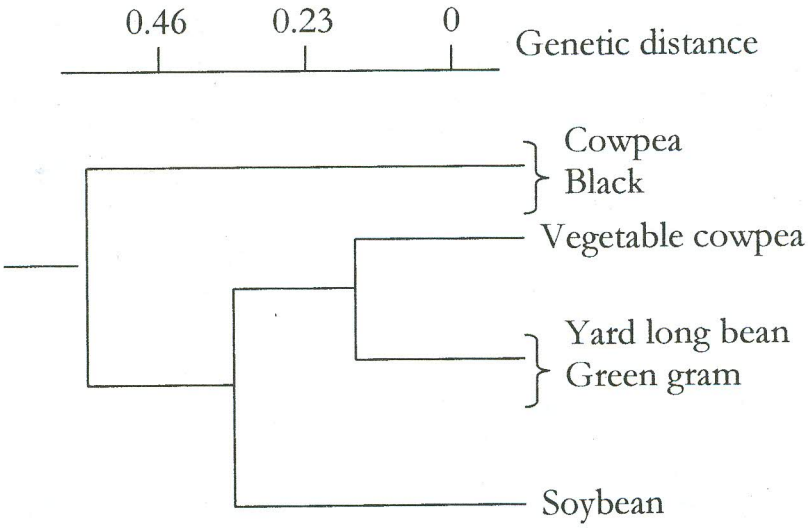

Figure 1. Dendogram Derived for Cowpea, Vegetable cowpea, Yard long bean, Green gram, Black gram and Soybean using Random Primers OPN 06, OPN 07, OPN 14, OPP 01, OPN 09 and OPN 20

Discussion

All rhizobial isolates could be grouped into four main clusters using the random primers OPN 06, OPN 07, OPN 14, OPP 01, OPN 09 and OPN 20. One cluster included isolates from cowpea and black gram and a second cluster included only vegetable cowpea. A third cluster comprised of yard long bean and green gram, whereas the fourth one comprises of soybean (Figure 1).

At a genetic distance of about 0.23, two clusters could be observed, which grouped vegetable cowpea and yard long bean, with green gram isolates in a single cluster (Figure 1.) Soybean isolates in another cluster were related to the above group at a genetic distance of about 0.35. All above isolates were related to cowpea and black gram at a genetic distance of about 0.60. This indicates that the isolates from black gram and vegetable cowpea, and yard long bean and green gram are more related to each other and share more DNA sequences among them than with the rest of the isolates.

Conclusion

Cowpea and black gram rhizobia can be considered genetically similar. Mae (yard long bean), mung bean (green gram) and vegetable cowpea are related, whereas, within the group, vegetable cowpea is distantly related to the rest. The rhizobial isolates of soybean are much distantly related to the isolates obtained from other crops.

The studied population of *Bradyrhizobium* showed high degree of polymorphism. However, this needs further confirmation by repeating RAPDs using a large number of primers.

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References

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