DETERMINATION OF THE GENETIC DIVERSITY OF SELECTED MANGROVE SPECIES USING RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD)

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Introduction

Mangroves have been unique amongst land plants in their ability to tolerate a wide range of salinities (Tomlinson, 1986). The mangrove forests in Sri Lanka are characterized by typical mangrove species such as *Rhizophora* spp., *Bruguiera* spp., *Avicennia* spp., *Sonneratia* spp., and *Excoecaria* spp. (Fortuna and Wilkie, 2003).

Mangrove ecosystems surrounding Sri Lanka have been increasingly facing serious threats of destruction due to non-sustainable human activities in an around the mangrove habitats (IUCN, 2005). The main objective of this study was to conduct a molecular analysis of commonly found mangrove species to determine their genetic diversity and thereby describe their genomic relationships. This knowledge would provide useful information for identification of different varieties, cultivars and ecotypes of mangrove species in the future conservation programmes.

Materials and Methods

The research was conducted at the Agricultural Biotechnology Centre (AgBC), Faculty of Agriculture, University of Peradeniya, Sri Lanka from late 2008 to mid 2009. Three and a half year old mangrove plant materials belonging to three species namely, *Rhizophora apiculata*, *Bruguiera cylindrica*, and *B. sexangula*, were collected from the National Aquatic Resources Research and Development Agency (NARA), Kadolkele, Negombo, Sri Lanka. The selection of three mangrove species was based on their wide distribution in Sri Lanka.

Ten plants from each mangrove species were randomly selected from a plant nursery having hundreds of plants for the molecular analysis. The DNA extraction (miniprep) from leaf material of mangroves was carried out using CTAB method described by Abeysinghe *et al.* (2000), which was modified by including 0.2% β – Mercaptoethanol in the extraction buffer while in the DNA extraction. Composite DNA samples for each mangrove species were made by pooling the genomic DNA extracted from ten representatives of the same species.

The DNA was subjected to PCR amplification (Acharya *et al.*, 2005) using 11 random primers (OPA 07, OPA 08, OPA 11, OPA 14, OPD 04, OPD 06, OPD 08, OPD 15, OPM 01, OPM 06 and OPM 09) following Agarose gel (1.5%) electrophoresis. Amplified fragments were scored for further analysis to construct a dendrogram based on similarity coefficients generated by the Un-

weighted Pair Group Method using Arithmetic Averages (UPGMA). The analyses were done using the computer software package NTSYS-PC.

Results and Discussion

The RAPD analysis resulted in nine primers showing polymorphic bands. However, five primers (OPD 06, OPD 08, OPD 15, OPM 01 and OPM 09) produced species specific fingerprints of three mangrove species (Figure 1

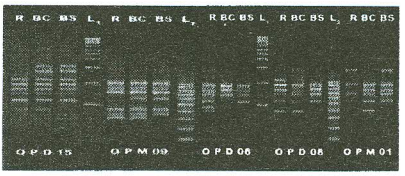

Figure 1. Species specific fingerprints of three mangrove species (R - *Rhizophora apiculata*, BC - *Bruguiera cylindrica*, BS - *Bruguiera sexangula*, L1 – 1 kb DNA Ladder and L2 – 100 bp DNA Ladder).

At the same time three primers (OPA 07, OPA 11 and OPA 14) reflected polymorphism only at generic level and also primer OPA 08 produced species specific finger prints of two *Bruguiera* species. However, out of the 11 primers used, two primers (OPD 04 and OPM 06) showed monomorphic banding profiles suggesting common DNA bands that are being shared among three different individuals in the same family.

The results revealed that among the three species studied, *B. cylindrica* and *B. sexangula* are more closely related being similar at 35.78% whereas *R. apiculata* shows a relatively distant relationship to the two *Bruguiera* species having similarity levels of 16.33% and 17.41%, respectively. According to the dendrogram, the clustering pattern showed grouping of the three selected mangroves into two different clusters. The two species of genus *Bruguiera* came under the same sub cluster justifying their inclusion under the same generic name while *R. apiculata*, which belongs to genus *Rhizophora*, separated from these *Bruguiera* species.

Conclusions

Among the 11 primers used, only five primers (OPD 06, OPD 08, OPD 15, OPM 01 and OPM 09) produced species specific fingerprints of the three mangrove species (*R. apiculata*, *B. cylindrica* and *B. sexangula*). Molecular analysis using RAPD markers demonstrated that the two *Bruguiera* species are much closely related while *R. apiculata* is slightly divergent from those two species.

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