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Genetic and geographic variation of the mangrove tree *Bruguiera* in Sri Lanka

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Abstract

The variation in two *Bruguiera* species in populations from different climatic and macrogeographic regions along the western to southeastern coastal area in Sri Lanka was studied using RAPD (Random Amplified Polymorphic DNA). Intra- and interspecific variation was observed using 20 primers of the 45 primers screened. Some primers showed diagnostic banding patterns and allowed unambiguous differentiation between *B. sexangula* and *B. gymnorhiza* without any indication of hybrid individuals. RAPD markers were also used to produce a similarity matrix among individuals of a species. A higher degree of polymorphism was observed within populations of *B. gymnorhiza* in Rekawa, situated in the climatically intermediate (between the wet and dry) zone, than within populations from the wet zone (Galle and Negombo). RAPD data analysis did not reveal a clear relationship between genetic differences and macrogeographical variation along the western and southern coastal area in Sri Lanka, but indicated that a small population of *B. sexangula* was genetically different. A more pronounced genetic difference at the population level in *B. sexangula* than in *B. gymnorhiza* is suggested.

Keywords: DNA; RAPD; Genetic diversity; *Bruguiera*; Sri Lanka; Mangroves

1. Introduction

Mangroves are a taxonomically diverse assemblage of woody plant communities belonging to several unrelated angiosperm families with special adaptations to saline conditions (De Silva and Balasubramaniam, 1985; Tomlinson, 1986). Although they are found in tropical areas world-wide, mangroves become more limited in their distribution in subtropical

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areas due to a lack of low-temperature resistance (Lindén and Jernelöv, 1980; Dodd et al., 1995). Though mangrove ecosystems occur in tropical as well as subtropical regions, the floristically richest mangroves are found in tropical southeast Asia (Lindén and Jernelöv, 1980).

Although ecological and physiological properties of mangroves are well understood, no attempts have been made to evaluate the potential values of using genetic markers of mangroves in Sri Lanka for conservation purposes. The identification of taxa, information on the geographic structure and a more precise understanding of the organisation of the existing genetic diversity within and among populations are very important for the conservation of genetic resources (Triest, 1991; Aldrich et al., 1992; Russell et al., 1993; Dawson et al., 1995, 1996). The mechanisms of gene flow and the distribution of genetic variation within and among populations have practical importance in terms of conservation strategy since both factors affect the genetic structure of the populations (Dawson et al., 1997). To our knowledge no studies have been conducted to examine how genetic variation in natural populations of *Bruguiera* species may vary across different geographic and different climatic areas either by biochemical or genetic (allozymes and DNA) markers.

Although it is possible to distinguish both species at a morphological basis, it remains difficult to identify individuals in mixed populations. Therefore an estimation and description of complex genetic relationships, e.g. possible hybridisation between species or introgression of the populations in the field is difficult using phenological and morphological descriptors. The use of molecular markers might enhance the understanding of such situations.

RAPD (Randomly Amplified Polymorphic DNA) which is a PCR based technique is attractive as it requires no prior knowledge of sequence and as a small amount of DNA (20 ng) is enough for an amplification (Williams et al., 1990). This method is relatively quick and economical for the assessment of genetic variation which is not measurable using morphological and allozyme methods. The molecular basis for RAPD has been discussed (Hadrys et al., 1992) and may provide either single base pair changes in the primer binding sites or deletion in the region between the primer binding sites. Although RAPDs are simple, their results are sometimes controversial because of a lack of reproducibility of some amplification products. Despite this drawback, RAPDs are used in population genetics studies. RAPDs are also criticised in population genetics because heterozygosity cannot be determined (Williams and St. Clair, 1993).

The objective of this paper is to use clearly amplified RAPD fragments to examine the genetic variation within and between populations of *B. sexangula* and *B. gymnorhiza* which originated from different geographic and climatic regions of Sri Lanka.

2. Materials and methods

2.1. Sample collection

The study area was chosen across the distribution of *B. sexangula* and *B. gymnorhiza* in contrasting climatic and different macrogeographic situations along the coastal belt of Sri Lanka. Leaf material was collected from individual trees of populations of *B. sexangula* and *B. gymnorhiza* in order to cover the contrasting climatic zones (warm lowland wet zone and

intermediate) and different geographic areas of the island. A total of five localities covered the coastal areas from the western to the southeastern coastal region of Sri Lanka. Climatically the wet zone covers the sampling sites Negombo (Locality 1, 07°11′N 79°50′E), Galle (Locality 2, 06°01′N 80°14′E), Dondra (locality 3, 05°96′N 80°34′E) and Kudawella (Locality 4, 06°30′N 77°30′E). Dondra and Kudawella are geographically close (15 km) in the wet zone in the island. Rekawa (Locality 5, 08°00′N 74°45′E) is located in the intermediate zone of the island (Müller-Dombois, 1968). These locations were selected in order to resolve maximal geographic variations within and between the species and populations of this genus.

2.2. DNA extraction and amplification

Total genomic plant DNA was extracted from young frozen leaf samples. Leaf tissue was ground to powder in liquid nitrogen using a mortar and pestle, and then 5 ml of extraction buffer was added. The extraction buffer consisted of 3% (w/w) CTAB (hexadecyl trimethyl ammonium bromide), 2M NaCl, 100 mM Tris–HCl pH 8.0, 1% β-mercaptoethanol, 20 mM EDTA, and 1% PVP (polyvinyl polypyrrolidone). The homogenate was incubated at 65°C for 30 min in a 50 ml centrifuge tube (Greiner); 5 ml of chloroform/isoamyl alcohol (24 : 1) was added and the mixture was centrifuged at $3000 \times g$ for 10 min The supernatant was treated with 10 ml of chloroform/isoamyl alcohol, centrifuged again for 10 min, precipitated by mixing with 0.6 volume ice-cold isopropanol, and then kept at room temperature for 15 min and pelleted by centrifugation at $7700 \times g$ for 15 min. The pellet was washed with 70% ethanol to get rid of salts, air dried and dissolved in sterile water. This was treated with 10 mg ml^{-1} RNase A (Sigma). The quality (contaminants of polysaccharides) of the DNA was monitored spectrophotometerically from 200 to 300 nm. The concentration of the DNA samples was estimated by a visual comparision of fluorescence after electrophoresis on 0.8% w/v agarose gels against known concentration of uncut lambda-DNA. Amplification was carried out according to Williams et al. (1990) and amplification products were size-fractionated on a 1% w/v agarose gel containing 10 μg ml^{-1} of ethidium bromide. They were visualised under UV light and photographed for further analysis.

2.3. Primer screening and data analysis

Fourty-five arbitrary oligonucleotide primers obtained from Operon technologies (USA) were screened using genomic DNA of *B. sexangula* and *B. gymnorhiza* to find out which of the primers could be used to differentiate the species. Reproducibility of the amplified products was tested, repeating the same reaction at least twice. Since RAPD markers are dominant, a particular DNA band (locus) which is generated from the genome of one individual, but absent of a second individual of the same species represents a polymorphism. In an attempt to identify species-diagnostic markers, samples from each species were amplified simultaneously and amplified products were run side by side on a same gel.

The amplification products obtained for all samples were compared to each other and screened for the presence/absence of specific markers. In order to assess the genetic structure within and among *Bruguiera* populations, all 64 diagnostic RAPD markers were analysed using NTSYS-pc (Rohlf, 1993) and Popgene (Yeh and Boyle, 1997). Relationships between

populations were analysed by constructing a pair-wise similarity matrix (SM) between individuals using a simple matching coefficient on the basis of presence/absence RAPD data. ($SM = 2n_{ab}/n_a + n_{b,}$ where n_a and n_b represent the total number of bands present in two individuals *a* and *b*, respectively, and n_{ab} is the number of bands which are shared by both individuals). The similarity matrix was then used as the basis for ordination by Principal Coordinate Analysis (PCO). Shannon's information index of diversity was calculated from the frequency of the RAPD bands within each population using the formula $H_0 = \Sigma p_i \ln p_i$ (Lewontine, 1973) where p_i is the frequency of a RAPD band. Genetic similarities were calculated between populations (Nei, 1978) for producing an UPGMA cluster (unweighted pair group method averaging).

3. Results

3.1. RAPD profile

Twenty primers which showed intra-and interspecific banding pattern for these species were used for further analysis of all 56 individuals of both species. Three categorised groups of amplified products of these species are listed in Table 1. A total of 64 different

Table 1
The number of primers used for DNA amplification of genomic DNA of *B. gymnorhiza* and *B. sexangula* and the number of amplified products generated from each primer

Primer		Number of polymorphic bands		
		Bg	Bs	Within both species
OPA-14	TCTGTGCTGG	01[a]	–	02
OPA-15	TTCCGAACCC	01[a]	–	01
OPE-07	AGATGCAGCC	02[a]	–	02
OPE-09	CTTCACCCGA	02	01	01
OPE-14	TGCGGCTGAG	–	–	01
OPE-15	ACGCACAACC	02[a]	–	01
OPE-16	GGTGACTGTG	01[a]	02[a]	–
OPE-18	GGACTGCAGA	01	01	02
OPJ-07	CCTCTCGACA	–	01[a]	01
OPJ-11	ACTCTCGACA	01[a]	01[a]	01
OPJ-12	GTCCCGTGGT	–	–[a]	02
OPJ-20	AAGCGGCCTC	–	01[a]	02
OP0-06	CCACGGGAAG	03[a]	01[a]	–
OP0-15	TGGCGTCCTT	02[a]	–	–
OPO-16	TCGGCGGTTC	02	–	02
OPT-06	CAAGGGCAGA	01	01[a]	01
OPT-07	GGCAGGCTGT	–	02	01
OPT-14	AATGCCGCAG	–	01[a]	03
OPT-15	GGATGCCACT	01	01[a]	01
OPT-16	GGTGAACGCT	01	01[a]	03
Total	20	21	14	29

[a] Intraspecific markers.

RAPD markers were amplified by 20 random primers. The number of bands for each primer which produced a polymorphic pattern varied from one (e.g. OPO-15 in *B. sexangula*) to 13 (e.g. OPE-09) and varied in size from about 0.5 to 3 Kb. Some populations showed clear differentiation between different geographical regions. For example, all individuals of *B. sexangula* of the Rekawa population lacked the 0.9 Kb fragment produced by primer OPE-07, whereas all the other individuals of both species possessed this fragment.

3.2. Discrimination and genetic variation of B. sexangula and B. gymnorhiza

A total of 56 individuals were screened for 20 selected primers.The primers OPA-14, OPE-09, OPE-16, OPE-18, OPJ-07, OPO-15 and OPT-07 resulted in a unique pattern for *B. gymnorhiza* and *B. sexangula* (Table 1). Amplification with primer OPO-15 showed a diagnostic and unique banding pattern for *B. gymnorhiza* and *B. sexangula*. OPO-15 primed the amplification of eight and one fragments of *B. gymnorhiza* and *B. sexangula*, respectively (Fig. 1). One fragment, (0.9 Kb) was present in both *B. gymnorhiza* and *B. sexangula* while all the other amplified fragments could only be observed in *B. gymnorhiza*. All the primers showed interspecific RAPD polymorphism while some primers showed intraspecific variation (Table 1).

The use of 64 RAPD loci and subsequent PCO and cluster analysis revealed that there were no hybrids between *B. sexangula* and *B. gymnorhiza* (Figs. 2 and 3). They were clearly separated into two distinct groups along the first coordinate axis which represents

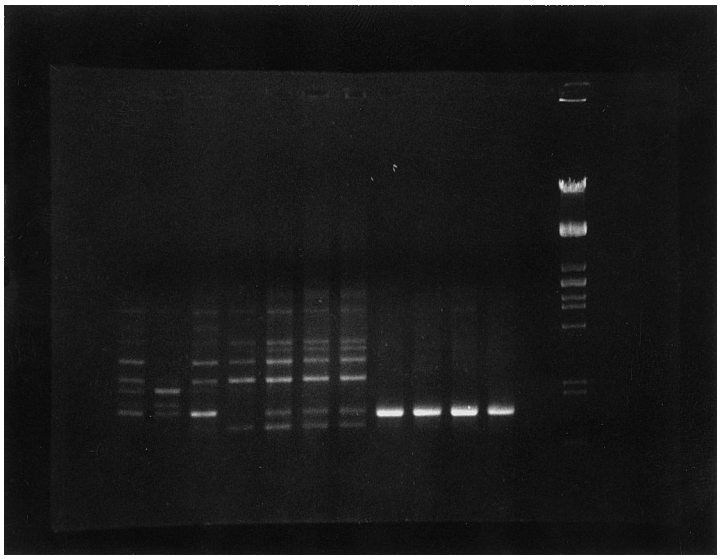

Fig. 1. Species diagnostic markers revealed by OPO-15 for *B. gymnorhiza* and *B. sexangula*. The 0.9 Kb (X) fragment is present in both species while other 7 RAPD products are present only in *B. gymnorhiza*. An arrow shows the generic marker for *B. gymnorhiza* (Lane 1–7) and *B. sexangula* (Lane 8–11), the negative control (Lane 12) and molecular weight marker (Lambda DNAdigested with Pst 1).

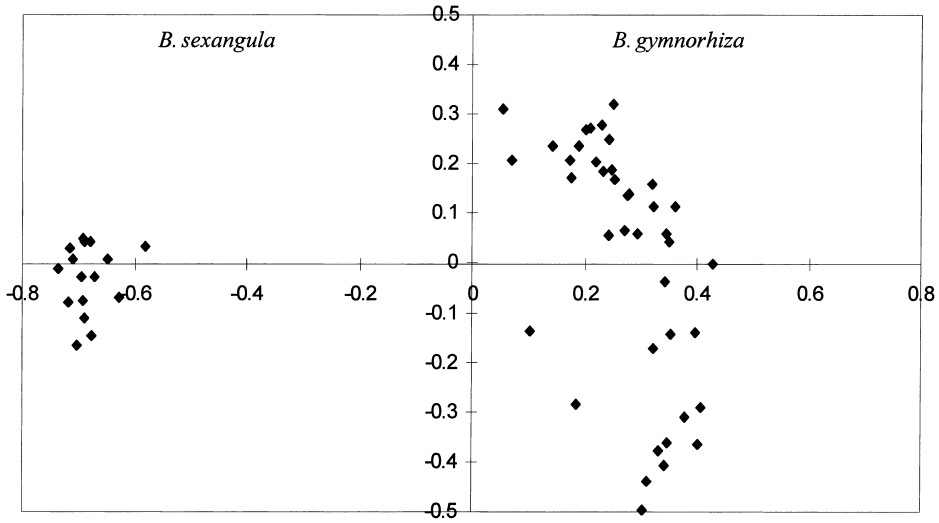

Fig. 2. Principal Coordinate analysis of individual *Bruguiera* trees. Axis 1 accounts already for 65% of the variation and separates the two species.

as much as 65% of the total variation (Fig. 2), and showed some differentiation among populations of *B. sexangula* and *B. gymnorhiza* originating from different geographic and contrasting climatic zones. The wide range of genetic similarity (65–97%) and derived Shannon's indices (Table 2) may emphasise the relatively wider diversity in the gene pool of *B. gymnorhiza*. The similarity coefficient for *B. sexangula* ranged from about 79 to 96 % and a more or less similar Shannon's index (Table 2) indicated a possible narrow genetic base for *B. sexangula* compared to *B. gymnorhiza*. The Dondra population exhibited the lowest genetic variability within population, but was a small sampling and small population size.

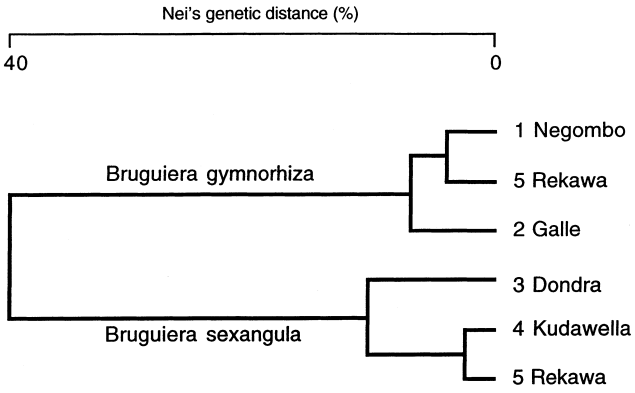

Fig. 3. UPGMA cluster of the *Bruguiera* populations indicates a low intraspecific differentiation.

Table 2
Locations and climatic zones of the island and number of individuals of invesigated *B. gymnorhiza* and *B. sexangula* for each population[a]

Species	Location	Climatic zone	Number of individuals	Shannon's index
B. gymnorhiza	Negombo	Wet	10	0.23
	Galle	Wet	08	0.24
	Rekawa	Intermediate	22	0.32
Within the species				0.37
B. sexangula	Rekawa	Intermediate	06	0.05
	Kudawella	Wet	06	0.13
	Dondra	Wet	04	0.08
Within the species				0.22

[a] Partitioning of genetic variation within the populations and within the species from different geographic areas of the country according to Shannon's index.

3.3. Geographic variation and genetic diversity

To examine the relationship between populations, further PCO analysis was undertaken for both species. For *B. sexangula*, the first principal coordinate effectively discriminated between the three populations. The individuals from populations Kudawella and Rekawa (Locality 4 and 5) from the wet and intermediate zone respectively were well segregated from the individuals of the small population from the wet zone (Locality 3), Dondra (Fig. 4). The distribution of individuals of *B. gymnorhiza* of the Rekawa population (Locality 5) overlaps with the individuals from Negombo and Galle (Locality 1 and 2, respectively).

A considerable amount of genetic variation within and between populations of *B. gymnorhiza* was detected by the 20 RAPD primers used in this study. The highest diversity values were thus for *B. gymnorhiza* in Rekawa followed by Negombo and Galle (Table 2).

4. Discussion

The successful identification of these two species using species-specific nuclear markers and only a limited set of primers proved to be promising for species detection. The DNA amplification fingerprinting technique allowed us to unambiguously differentiate *B. gymnorhiza* and *B. sexangula*. Although these two species have some similar morphologies (leaf morphology, leaf length, the colour of the petiole, the colour of the midrib), their RAPD fingerprinting differed markedly. These two species were well separated along the first axis of the PCO plot and cluster analysis suggested that RAPD data and morphological data are positively correlated.

Using the Shannon's index, the average RAPD diversity for *B. gymnorhiza* and *B. sexangula* were calculated as 0.37 and 0.23, respectively, indicating that *B. gymnorhiza* showed more genetic diversity than *B. sexangula*. The lowest diversity index 0.08 may be biased by the low sampling size in Dondra. The highest genetic diversity index, 0.32 was observed within the *B. gymnorhiza* population where the largest number of individuals were collected and originated from an intermediate zone sampling site, Rekawa. Individuals of

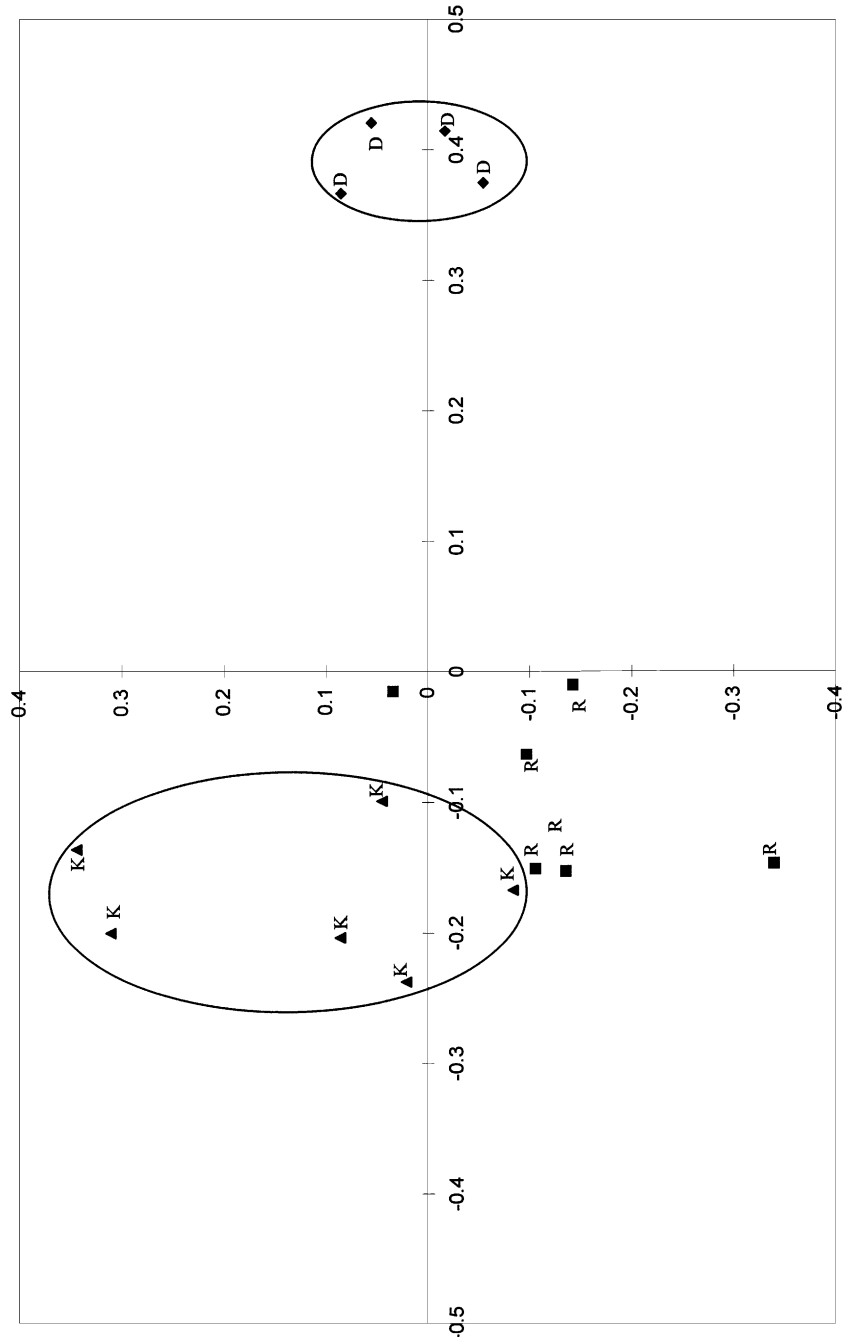

Fig. 4. Principal Coordinate analysis based on 64 RAPD markers within *B. sexangula* indicates a differentiation among the populations D-Dondra, K-Kudawella and R-Rekawa.

B. gymnorhiza from the Rekawa population exhibited a wider range of variability compared to the individuals from Negombo or Galle. In this mixed population (Locality 5, Rekawa) no genetic hybrids or intermediates of *B. sexangula* and *B. gymnorhiza* could be observed. Significant differences of the genetic diversity of populations of *B. gymnorhiza* and *B. sexangula* from wet or intermediate zones of the island were not observed.

The frequency differences of RAPD exhibited among individuals of *B. sexangula* revealed in a polarisation of genetic variation into two groups along the first axis. The Dondra population (Locality 3) was clustered separately from the other two *B. sexangula* populations. Individuals from Rekawa and Kudawella populations are genetically more similar. Although our sampling of *B. sexangula* from Dondra was small, all four individual trees separated from other populations of *B. sexangula*. Rekawa and Kudawella sampling sites are separated from each other by only 18 km, but show different climatic, geographical and ecological parameters. Therefore analysis of RAPD data could be useful to detect differentiation among *B. sexangula* populations which might be related to geographical regions.

Despite the great interest in management and conservation of tropical tree species, a very limited number of researches have been conducted on the population genetics of tree species (Dawson et al., 1996; Parani et al., 1997; Schierenbeck et al., 1997). However studies on genetic diversity within populations of woody species showed that a high level of genetic diversity mostly resides within the populations (Hamrick et al., 1992). In our study, partitioning of variation showed that most of the variation was detected within (70%) the populations of *B. gymnorhiza* but for *B. sexangula* 60% of the variation was observed among populations. For *B. gymnorhiza* this finding corresponds to allozyme (Hamrick et al., 1992) and RAPD data (Schierenbeck et al., 1997) indicating that for woody species most of the variation is maintained within the population. The latter result is similar to the genetic variation of a tropical tree species *Gliricidia sepium* where 60% of the variation was found among populations (Chalmers et al., 1992).

The differentiation of these populations could partly be explained as a result of abiotic (geographical, e.g. hydrographic connections and/or climatic differentiation, e.g. annual rainfall differences) and biotic (pollination between populations, seed dispersal etc). Since the genetic variability in the mixed population of Rekawa is somewhat higher, a possible introgression could have occurred. Whether these environmental conditions may have affected and selected the higher genetic variation of *B. gymnorhiza* of the Rekawa population located in the intermediate zone is not known but could now be put forward as a working hypothesis.

Within and among the *Bruguiera* populations studied here, RAPD revealed a significant degree of geographically related (locality based) differentiation even within the small scale distance, e.g. Kudawella, Rekawa and Dondra. Our results showed already certain trends in diversity which might not be evident in sampling only a few individuals per site and which clearly deserve further analysis. Genetic variation within each of the populations of *Bruguiera* may vary with the population size. Therefore, comparing the small forest area at Dondra to the large populated area of Negombo should be carried out. Much remains to be studied using larger sampling sizes since the current sampling was too limited to support far-reaching conclusions on the diversity among and between populations of *Bruguiera*.

In conclusion, this study demonstrated that decanucleotide DNA fingerprinting can be an effective and useful method to distinguish individual genotypes, to assess and to delineate

the pattern of genetic variation within and among populations of *B. sexangula* and *B. gymnorhiza*. For management and conservation purposes, the identification of *B. sexangula* and *B. gymnorhiza* and their putative hybrids or intermediates, even at an early stage of their life cycle, e.g. seedling stage, could be accomplished using RAPD markers.

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