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T.L. Maguire · K.J. Edwards · P. Saenger · R. Henry

Characterisation and analysis of microsatellite loci in a mangrove species, *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae)

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Abstract An enriched microsatellite library of the mangrove species *Avicennia marina* was constructed, in which 85.8% of the clones contained microsatellite sequences. Of the microsatellite repeat sequences isolated, 55.0% were di-nucleotides, 34.2% were tri-nucleotides, 50.0% were perfect, 24.2% were imperfect, and 15.0% were compound. Four different di-nucleotide repeats were isolated with repeat lengths ranging from 5 to 33; ten different tri-nucleotide repeats were isolated with repeat lengths ranging from 3 to 25. The most common di-nucleotide was the AC/TG repeat; the most common tri-nucleotide was the CCG/GGC repeat. Sixteen microsatellite sequences were selected for primer design, and 6 primers were selected to investigate the polymorphism detected among 15 individuals of *A. marina* from three natural populations in Australia. A total of 40 alleles were detected at 6 microsatellite loci. The number of alleles per microsatellite locus ranged from 5 to 13. On average, 7 alleles were detected per locus. All microsatellite loci showed high levels of gene diversity (heterozygosity), with values ranging from 0.53 to 0.88; the mean value of gene diversity was 0.70. Microsatellite loci were also tested for conservation across *Avicennia* species. There was a decline in amplification success with increasing divergence between *Avicennia* species. The results indicate that microsatellites are abundant in the *Avicennia* genome and can be valuable genetic markers for assessing the effects of deforestation and forest fragmentation in mangrove communities, which is an important issue for mangrove conservation and afforestation schemes.

Key words *Avicennia marina* · Microsatellite · Mangrove · Genetics

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T.L. Maguire (✉) · P. Saenger
Centre for Coastal Management, Southern Cross University,
PO Box 157, Lismore, NSW, 2480, Australia
e-mail: tmaguire@scu.edu.au
Fax: +61 02 66222080

T.L. Maguire · R. Henry
Centre for Plant Conservation Genetics,
Southern Cross University, PO Box 157, Lismore, NSW, 2480,
Australia

K.J. Edwards
IACR-Long Ashton Research Station,
Department of Agricultural Sciences, University of Bristol,
Long Ashton, Bristol, BS41 9AF, UK

Introduction

Microsatellites, also known as simple sequence repeats (SSRs), are short (1–5 bp in length) tandemly repeated DNA sequences, the most abundant being the di-nucleotide repeat. Microsatellites are an important class of marker because of their abundance and hypervariability. They occur frequently and randomly in eukaryotic genomes (Tautz and Renz 1984) and are highly informative markers (Weber 1990; Weber and May 1989). Weber (1990) categorised microsatellites as perfect repeats (without interruption), imperfect repeats (interrupted by non-repeat bases), and compound repeats (two or more repeat runs present adjacent to each other). Variation in the number of repeats among different genotypes provides the basis for polymorphism, which can be used in plant genetic studies (Condit and Hubbell 1991). Microsatellite loci can be highly polymorphic between species, and between individuals and populations within a species. Also, microsatellites can be easily distributed between laboratories as primer sequences, thus providing a common tool for collaborative research by acting as universal genetic markers.

Microsatellites can be isolated directly from total genomic DNA libraries, or from libraries enriched for specific microsatellites. Alternatively, microsatellites can be found by searching public databases such as GenBank and EMBL. The first report of the isolation and cloning of plant microsatellites was in a tropical tree species (Condit and Hubbell 1991). Although there is less data on plant than animal microsatellites, there are already

some important differences. Overall, there is a tenfold reduction in the frequency of di-nucleotide repeats detected in plants compared to humans (Powell et al. 1996); but in plant genomes the AT/TA repeat predominates, compared to the AC/TG repeat in humans; these features tend to distinguish the plant and animal genomes. The relatively low frequency of microsatellites in plant genomes presents some technical problems for the large-scale isolation of microsatellites. Furthermore, the most common di-nucleotide (AT/TA) repeat in plants is difficult to isolate from libraries because of the palindromic nature of the repeat sequence.

Standard methods for the isolation of plant microsatellites involve the creation of a small-insert genomic library, library screening by hybridisation, DNA sequencing of positive clones, primer design, locus specific polymerase chain reaction (PCR) amplification, and the identification of polymorphisms. In order to improve the efficiency, microsatellite-enriched libraries are developed using various methods, with selection either before or after library construction. Pre-cloning enrichment methods, such as that of Edwards et al. (1996), involve the fragmentation of the genomic DNA by endonuclease digestion, followed by the ligation of adapters to create DNA fragments with defined sequences at both ends. Microsatellite-containing fragments are then enriched by hybridising to membranes with bound oligonucleotides and fragments are then isolated, size-selected, and cloned. The sequences flanking each microsatellite are highly conserved between individuals and can be used for designing suitable PCR primers for amplification of the loci.

Microsatellites are now being described in many plant species, but information on microsatellites in any particular plant species is limiting. Microsatellites have been reported in several forest tree species such as: *Pinus* (Smith and Devey 1994; Kostia et al. 1995; Echt et al. 1996), *Quercus* (Dow et al. 1995), *Picea* (van de Ven 1996), *Pithecellobium* (Chase et al. 1996), *Swietenia* (White and Powell 1997), and *Eucalyptus* (Brondani et al. 1998). All these studies, however, resulted in the development of only a small number of markers; typically less than ten. There is no information on microsatellite markers for mangrove species.

Mangrove forests are a feature of the intertidal zone of the tropical and subtropical coastlines of the world. They comprise some 80 higher plant species in several taxonomic groups. About half of these species occur in Australia, which is believed to be near the centre of mangrove evolution (Hutchings and Saenger 1987). Mangrove forests all over the world are heavily exploited for wood and fish-pond operations, as well as other activities. The exploitation of mangroves has resulted in the loss of genetic diversity of mangrove ecosystems, as well as the loss of valuable wood resources. Mangrove reserves, afforestation schemes, and enrichment plantings have been established to overcome these losses. The conservation of mangroves is, thus, a major priority in coastal areas of many countries.

Avicennia marina (Forsk.) Vierh. is an important mangrove species because it can grow and reproduce across a wide range of climatic, saline, and tidal conditions. It is the most widely distributed of all mangrove tree species. *A. marina* is found in the Indo-West Pacific and ranges in latitude from 25°N to 38°S. The structure of *A. marina* stands varies from gnarled shrubbery on dry coastlines and coral atolls, to closed estuarine forests up to 40 m tall within larger systems of wet coastal tropical regions (Duke et al. 1998). The objectives of study described here were to: (1) develop microsatellite markers for *A. marina* from an enriched microsatellite library, (2) to determine the frequencies of different microsatellite repeats in the *A. marina* genome, and (3) to discuss their potential for application in the conservation of mangrove genetic resources, population genetics, and plant breeding.

Materials and methods

Plant material and DNA isolation

Plant material of 1 individual of *A. marina* at North Creek, a tributary of the Richmond River, in Ballina, NSW, was collected for the enriched microsatellite library construction. Fifteen individuals from three natural populations in Australia (NT, NSW, WA), representing three widely separated populations of *A. marina*, were used for the investigation of polymorphism detected by microsatellites. Total genomic DNA was isolated from leaf tissue using a modified CTAB method (Maguire et al. 1994).

Construction of an enriched microsatellite library of *A. marina*

The procedure for construction of the enriched microsatellite library was as described by Edwards et al. (1996). The following oligonucleotides were used to enrich for DNA fragments containing microsatellites: $[CT]^{15}$, $[CA]^{20}$, $[ACT]^{14}$, $[AGA]^{14}$, $[CAA]^{14}$, $[CTA]^{14}$, $[CTT]^{14}$, $[CTG]^{10}$, $[CAG]^{10}$, $[GAC]^{14}$, $[AGC]^{14}$, $[CAT]^{14}$, $[ACA]^{14}$. Aliquots of 200–400 ng of genomic DNA were digested with *Rsa*I and *Alu*I separately. An *Mlu*I adaptor (consisting of a 21-mer and a 25-mer primer) was ligated to the digested fragments. Enrichment for microsatellites was carried out by hybridisation to a single Hybond N^+ filter with bound oligonucleotides. Following hybridisation, the filter was washed, and bound DNA fragments were eluted. The eluted DNA was amplified by PCR using the 21-mer adaptor primer. The enriched DNA was then digested with *Mlu*I, size selected, and ligated into a modified pUC19 vector (pJVI) containing a *Bss*HI site (K.J. Edwards, unpublished). Plasmids were then transformed into competent cells (DH10B Life Technologies) and plated onto LB-agar plates containing ampicillin and X-gal. Plasmids from individual colonies were prepared using a Boehringer Mannheim High Pure Plasmid Isolation Kit and sequenced from the M13 primer sites using the BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems) with automatic analysis on a Applied Biosystems Model 377 sequencer.

PCR primer selection and synthesis

PCR primers from the unique sequence flanking the microsatellite loci were designed using the computer program MacVector 6.0 (Oxford Molecular Limited). Selection criteria were based on melting temperature, base composition, and secondary structure formation. The primers were synthesised commercially by Pacific Oligos Pty. Ltd. The primer pairs were tested against 4 individuals, one from each of the three different populations of *A. marina*,

and the original individual from which the library was constructed (as a positive size control).

PCR amplification of the microsatellite loci

The PCR amplification conditions were: 1×reaction buffer (10 m*M* TRIS-HCl, 50 m*M* KCl, pH 8.3, Boehringer Mannheim), 1.5 m*M* $MgCl_2$, 200 µ*M* each dNTP, 0.2 µ*M* each primer, 1 U *Taq* polymerase (Boehringer Mannheim), and 10–50 ng genomic DNA, in a total volume of 25 µl. After an initial heating step at 94°C for 3 min, samples were incubated for 30 cycles of 94°C for 30 s, 60°C–55°C for 30 s, and 72°C for 1 min. The reactions were completed by incubating at 72°C for 5 min and held at 4°C. Cycling was on a Perkin Elmer Cetus 9700 Thermal Cycler. The PCR products were separated using three electrophoretic methods: (1) 2% agarose gels run at 80 V for 2 h in 1×TBE buffer and stained with ethidium bromide, (2) non-denaturing 6% polyacrylamide gels run at 250 V for 2.5 h in 1×TBE buffer and visualised by silver staining, and (3) PCR products were fluorescently labelled (FAM, TET, HEX), then denatured for 3 min at 95°C and separated by capillary electrophoresis on a PE Applied Biosystems 310 Genetic Analyser, followed by analysis with GENESCAN™ and GENOTYPER™ software (PE Applied Biosystems).

Data analysis

Gene diversity (heterozygosity) was calculated according to Wier (1990). Thus:

$$\text{gene diversity} = 1 - \sum P_{ij}^2 .$$

P_{ij} is the frequency of the *j*th pattern for the microsatellite marker *i* and is summed across *n* patterns. Anderson et al. (1993) suggest that gene diversity is the same as the polymorphism information content (PIC).

Conservation of microsatellite loci across *Avicennia* species

Four species from the genus *Avicennia* were tested for microsatellite conservation. Samples of *A. germinans* were collected from Benin, West Africa; *A. officinalis*, *A. alba*, and *A. rumphiana* were collected from Pandang Reservoir, Singapore. Total genomic DNA was isolated from leaf tissue, and microsatellite loci were PCR-amplified as described previously. Six polymorphic microsatellite loci were tested. The PCR products were separated on 3% agarose gels run at 120 V for 2 h in 1×TBE buffer and stained with ethidium bromide. To confirm the identity of cross-species amplification products, PCR products from homozygous individuals at one locus were purified using the Promega Wizard PCR Preps Kit and then sequenced using both the forward and reverse primer with a BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems). Sequencing products were then automatically separated on a Applied Biosystems Model 377. Resulting sequence data were then edited using SeqEd (PE Applied Biosystems) and aligned using the PILEUP program of the GCG (The University of Wisconsin Genetics Computer Group) computer software.

Results

Isolation of microsatellite sequences in *A. marina*

Of the 120 clones sequenced, 85.8% contained microsatellite sequences, and 20.0% contained more than one microsatellite sequence (Table 1). The insert size of the clones ranged from 104 bp to 683 bp, as determined by sequence analysis. The average insert size was 265 bp.

Table 1 Frequency of microsatellite sequences in the enriched *A. marina* microsatellite library

Clones containing a microsatellite sequence	85.8%
Clones containing >1 microsatellite sequence	20.0%
Clones containing suitable flanking sequence	67.5%
Insert size range	104–683 bp
Average insert size	265 bp
Di-nucleotide repeats	55.0%
Tri-nucleotide repeats	34.2%
Perfect repeats	50.0%
Imperfect repeats	24.2%
Compound repeats	15.0%

Table 2 Frequency (as a percentage of the total number of clones containing a microsatellite) of different microsatellite sequences in the enriched *A. marina* microsatellite library

Type of microsatellite	Frequency (%)	Average repeat length (bp)
AC/TG	40.8	18
AG/TC	5.0	33
AT/TA	9.1	5
GC/CG	0.8	5
AAG/TTC	0.8	7
CAC/GTG	0.8	3
CAG/GTC	1.6	16
CCG/GGC	13.3	7
CGC/GCG	8.3	7
CGG/GCC	3.3	8
CTG/GAC	1.6	14
GCT/CGA	1.6	25
TGG/ACC	0.8	5
TTG/AAC	0.8	5

Of the microsatellite sequences isolated, 55.0% were di-nucleotide repeats, 34.2% were tri-nucleotide repeats, 50.0% were perfect repeats, 24.2% were imperfect repeats, and 15.0% were compound repeats. Of the clones containing microsatellites, 67.5% had suitable flanking regions for primer design.

Four different di-nucleotide repeats were isolated with repeat lengths ranging from 5 to 33 (Table 2); ten different tri-nucleotide repeats were isolated with repeat lengths ranging from 3 to 25. The most common di-nucleotide was the AC/TG repeat, which had a frequency of 40.8% (Table 2); the next most common di-nucleotides were AT/TA and AG/TC, with frequencies of 9.1% and 5.0%, respectively. The most common tri-nucleotides were CCG/GGC, CGC/GCG, and CGG/GCC, with frequencies of 13.3%, 8.3%, and 3.3%, respectively.

To date, sequence comparison of enriched clones from various libraries have shown no evidence for the selective enrichment of specific microsatellite sequences. However, we have identified clones containing microsatellites which were not bound to the Hybond N^+ membrane, and can account for only half of the bound microsatellite sequences. This suggests that many more different microsatellite sequences probably exist within the genome of *A. marina*. Interestingly, a significant number of clones (20.0%) contained two or more different microsatellite sequences.

Table 3 Characterisation of 16 *A. marina* microsatellite loci

Locus	Primer sequences (5´-3´)	Repeat type	T^m (°C)	Expected size (bp)
M3	GGTTCCTGCAAGTATGTCAACACCCTC ACCTCGATTCCTCCCCGAATGC	$(TG)^{15}$	60	182
M13	CAATGGTGATTCTCCAAAATTGCTTTG TGGTGAATAGATGACAGTAAGGATCAGCC	$(AT)^{10}(GT)^{12}$	59	192
M27	GGTGGAGTTTCAGTTCATCGTTCG CCGCAGTGGGGTTCATCAAAC	$(CCG)^{8}$	56	120
M32	TGTGAACTTTGCTTCAGAGTCTCGAAGATG AGTCAAATGGAGCCTCATTCCTCCG	$(AC)^{14}$	60	163
M34	TCTGCTGTTGCTGTTGTTGTTGATGC TGGTGTTGAAGACTAATCATGTGTTTCGC	$(GCT)^{14}$	60	183
M40	CCCATAGATGACGGCAATCTTATGATCC ACCATCCAAAATAAAATAAATCTCCCTCCC	$(AG)^{32}$	60	161
M47	TGACACCAAGGGAAATCAACATGCC GAACCTAGCGACCAATAGATCATCCTGG	$(CA)^{13}$	60	172
M49	TTTCCTCACGACAGACTAGAAACCACC CAATAAACTTGGATAAAGGCAACTCCGAC	$(TG)^{16}$	58	189
M62	TTGAGGAAAACATGGGACTTTCACTCG GTGGGAGTAGCCGCATAGAGTCACG	$(CGC)^{8}$	60	240
M64	CAAACCCTACCAATCAGAACACTTCAAGC CGATATTTGGCTAATCCACTCTGCTGACTG	$(CAG)^{8}$	60	156
M73	TTCCACAATCACTTGACCCTCGTCC TCTTCACAGGTCCTCTCCTGCCCTG	$(TG)^{15}$	60	176
M75	TCCATAATCAAACAACTCGACAACGAAATC TCTTCTCTCCCTATTCCAAACTGGCTTG	$(TG)^{14}$	60	207
M76	GCATGTTTCAGCCTCTTTGGTGCC CTTCCAAGTGGGATGCTCTTTGTCG	$(CA)^{25}(TA)^{3}$	60	168
M81	GAATGATGATCGGATGTTGCTACTCCTG CAATCCCAAAGCCCCAAAAATAATCC	$(CA)^{9}(CT)^{16}$	60	164
M85	TGACAGAGGTTTAGAGACATGGAGGGTGAG TGCCTCCCACATTCACCACACTGC	$(GGC)^{8}$	60	118
M98	CCCAAACTCGTTACGATGGATGACTTC CTTACAGTTGCGGTAAAATGAGACGTGC	$(CGG)^{8}$	60	228

Characterisation of selected microsatellite sequences

From the large number of microsatellite sequences isolated, 16 were selected for primer design (Table 3), and 6 primers were selected to investigate the polymorphism detected among 15 individuals of *A. marina* from three distinct natural populations in Australia. The original individual used in the library construction was also included as a positive size control. All of the amplified products were in the expected size range. Of the 6 primers tested over 15 individuals, three were di-nucleotide repeats, two were tri-nucleotide repeats, and one was a compound repeat (Table 4). The amplification products for all 6 loci were polymorphic in the material tested. A total of 40 alleles were detected at the 6 microsatellite loci. The number of alleles per microsatellite locus ranged from 5 to 13. On average, 7 alleles were detected per locus. All microsatellite loci showed high levels of gene diversity (heterozygosity), with values ranging from 0.53 to 0.88 (Table 4); the mean gene diversity was 0.70. Figure 1 shows an example of microsatellite alleles amplified in 3 individuals of *A. marina*, visualised using the PE Applied Biosystems 310 Genetic Analyser.

Table 4 Results of analysis of 6 microsatellite loci in 15 *A. marina* individuals

Locus	Microsatellite type	Gene diversity	Number of alleles
M3	$(TG)^{15}$	0.82	5
M40	$(AG)^{32}$	0.88	13
M47	$(CA)^{13}$	0.68	5
M64	$(CAG)^{8}$	0.53	5
M81	$(CA)^{9}(CT)^{16}$	0.53	6
M98	$(CGG)^{8}$	0.78	6

Conservation of microsatellite loci across *Avicennia* species

Within the source species, *A. marina*, each microsatellite primer pair produced a single, strong PCR product (Table 5). Amplifications from non-source species were sometimes weaker than those from the source species, but all products were single bands with no spurious amplification products. Primer pair M3 failed to amplify any non-source species; M40 amplified a weak product in *A. officinalis*; M47 amplified strong products in *A. alba*, *A. rumphiana*, and *A. officinalis*; M64 amplified weak products in all non-source species; M81 amplified strong products in *A. alba* and *A. rumphiana* and weak products in *A. officinalis* and *A. germinans;* M98 amplified strong products in *A. alba*, *A. rumphiana*, and *A. officinalis*. All primer pairs produced amplification products in the expected size range.

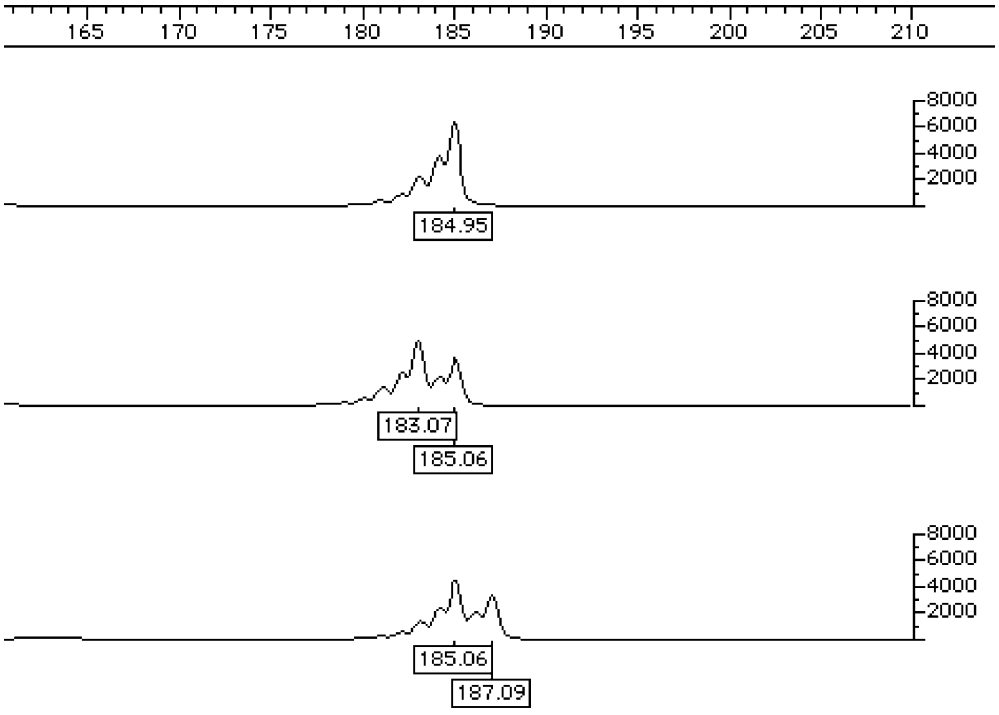

Fig. 1 An example of microsatellite alleles amplified from 3 individuals of *A. marina*, visualised using the PE Applied Biosystems 310 Genetic Analyser, then imported into GENOTYPER™ software for allele sizing. The *horizontal scale* represents the size in basepairs. The *vertical scale* represents the fluorescence intensity

Table 5 Results of PCR amplifications across five species of *Avicennia* for six microsatellite primer pairs polymorphic in *A. marina*

	M3	M40	M47	M64	M81	M98
A. marina	+[a]	+	+	+	+	+
A. alba	–	–	+	±	+	+
A. rumphiana	–	–	+	±	+	+
A. officinalis	–	±	+	±	±	+
A. germinans	–	–	–	±	±	–

[a] +, strong amplification product; ±, weak amplification product; –, no amplification product

Amplification products from homozygous individuals of non-source species were sequenced directly to confirm their identity and to examine whether the flanking sequences were conserved in length. Sufficient flanking sequences were obtained to confirm that each of the amplification products were homologous across species. Figure 2 provides clear evidence of insertions, deletions, and substitutions in the flanking sequence of the non-source species relative to the source species *A. marina*. Thus, microsatellite alleles of the same length in different species do not necessarily have the same number of repeats. Across-species allele lengths can differ by non-repeat unit lengths, which indicates changes in the flanking region as well as in the copy number of the repeats. Those primers which were successful in amplifying across all *Avicennia* species tested were tri-nucleotide repeats or compound repeats.

Discussion

We have successfully isolated microsatellites from an enriched library of *Avicennia marina*, in which most (85.8%) of the clones contained a microsatellite sequence. The frequency of each class of microsatellite is highly variable among plant species (Wang et al. 1994); our results indicate that AC/TG repeats appear to be well-dispersed throughout the genome of *A. marina*. This differs from many plant species surveyed in which AG/TC repeats are more abundant then AC/TG repeats (Powell et al. 1996). However, a recent survey of di-nucleotide repeat frequencies in pine trees revealed a greater abundance of AC/TG than AG/TC repeats (Echt and May-Marquardt 1997). Although less abundant than di-nucleotide repeats, tri- and tetra-nucleotide repeats are seen as desirable, since variation in the number of core units is easier to detect due to the larger repeat unit length. This permits a less ambiguous allele sizing and reduced stuttering when compared to di-nucleotide repeats (Edwards et al. 1991). Our results indicate that tri-nucleotide repeats (such as CCG/GGC and CGC/GCG) are relatively frequent in the *A. marina* genome, with frequencies of 13.3% and 8.3%, respectively, and may be valuable alternatives to di-nucleotide repeats. No precise estimates of the frequency of microsatellites in the *A. marina* genome can be made from our work, as the development of the microsatellite loci was based on a highly enriched library. The high efficiency of recovery of microsatellite-containing clones from the genomic library constructed with two different restriction enzymes and different classes of repeats suggests that there are many different types of microsatellite repeats available in the *Avicennia* genome for marker development.

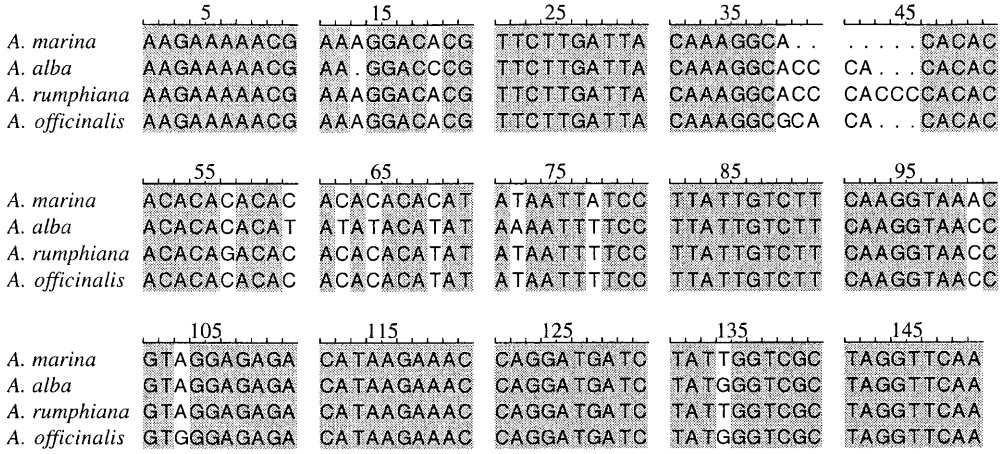

Fig. 2 Partial sequences of PCR products at the homologous microsatellite loci M47 of four species of *Avicennia*, showing homology of products and the presence of insertions, deletions, and substitutions in flanking regions. *Stops* indicate gaps and *shading* highlights base conservation in all four species. All sequences begin approximately 10 bases downstream of the appropriate primer

Microsatellite variation has been found to be generally high in many plant species. Although the number of alleles detected in a study is dependent on the sample size, it does give an indication of the degree of variability detected with microsatellite loci. In our study 6 microsatellite loci were examined and found to be highly polymorphic, with gene diversity estimates ranging from 0.53 to 0.88. However, these estimates are based on only 15 individuals; larger numbers of individuals will provide more accurate estimates of gene diversity. The current estimate is sufficiently high to indicate that microsatellites will be very useful for genetic analysis of *A. marina* and related species.

The transferability of microsatellite loci across *Avicennia* species means that polymorphic markers identified in *A. marina* as being useful for genetic analysis can also be useful in related species. In plants, the conservation of microsatellite loci has been observed between sub-species and between closely related species (Wu and Tanksley 1993; Morgante et al. 1994). The ability to use the same microsatellite loci in different plant species depends on the extent to which the flanking regions are conserved between related species and the stability of the microsatellite loci over time. Our results indicate a decline in amplification success with increasing divergence between *Avicennia* species. Out of the 6 loci, 4 amplified products in *A. marina*, *A. alba*, *A. rumphiana*, and *A. officinalis*, indicating a close relationship between these species. However, only 2 of the loci amplified products in *A. germinans*, indicating that *A. germinans* is more distantly related to *A. marina*. This is in agreement with a phylogenetic analysis of *Avicennia* based on morphological characters (Bousquet-Melou 1996).

In cross-species studies of other plants, insertions and deletions in the flanking sequence, as well as variation in the microsatellite repeats themselves were found (Peakall et al. 1998; Westman and Kresovich 1998). This flanking sequence variation can contribute to the increased number of alleles observed across species. Further analysis is therefore needed to correct for this effect in order to make inferences between species. This same observation argues against the use of microsatellites for phylogenetic analysis because alleles of the same length may not be homologous. In addition, the length of the PCR product may bias the number of allelic states, as longer flanking sequences increase the chance of finding genetic variation in cross-species comparisons.

Microsatellites are an efficient means of individual identification and can provide high resolution and sensitivity for the estimation of genetic parameters essential for assessing the effects of deforestation and forest fragmentation in mangrove communities. The need to understand what determines the patterns of genetic variation is an important issue for mangrove conservation and afforestation schemes. Most species which occupy environmentally sensitive habitats in both temperate and tropical regions, such as mangroves, are out-breeders. For these species, information on the extent of gene flow, genetic differentiation, and levels of inbreeding (particularly in fragmented populations) has important practical relevance for the conservation of genetic resources. Microsatellites have great potential in this respect; their multi-allelic, co-dominant nature allows individuals to be uniquely genotyped so that gene flow and paternity can be established. To date, microsatellites have revealed more polymorphism than other markers (Powell et al. 1996). The resolving power of microsatellites has been demonstrated in many plant species: for example, in a tropical tree species 4 polymorphic microsatellite loci were evaluated in two populations (Chase et al. 1996). A comparison of microsatellites with isozyme data of the same material revealed that the number of alleles, and the expected heterozygosity, of the microsatellite loci was more than double that obtained with 6 polymorphic isozyme loci.

To date, isozymes, restriction fragment length polymorphisms (RFLPs), and random amplified polymorphic DNA (RAPDs) have been used to characterise *Avicennia* species (Duke et al. 1998; Parani et al. 1997). Though useful in selected applications, isozymes, RFLPs, and RAPDs have some limitations. For example, it may be difficult to detect the state of a locus (heterozygote or homozygote) or to identify allele relationships. In addition, there are technical difficulties when dealing with

large numbers of samples, such as discrimination, sensitivity, reproducibility, the need for further analysis, as well as time considerations (Kresovich et al. 1995).

The ease of using microsatellite markers makes their use preferable, particularly when analysing large numbers of individuals with small numbers of loci. The use of fluorescence detection techniques further improves the analytical resolution of the amplified fragments, expedites data collection, and therefore reduce costs. High-resolution, high-throughput genetic analysis will provide the framework for the effective maintenance and utilisation of plant genetic diversity. Therefore, the molecular markers must be accessible, reliable, easy to use, and cost-effective. Due to their inherent attributes, microsatellites will be readily employed for these purposes. This will support the improved conservation and sustainable management of our ever declining mangrove genetic resources.

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