Diagnosis of interspecific hybrids between *Acacia mangium* and *A. auriculiformis* using single nucleotide polymorphism (SNP) markers

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(Received 4th November 2009)

Abstract

This paper describes a diagnostic system to verify interspecific hybrids between *Acacia mangium* and *A. auriculiformis* using single nucleotide polymorphism (SNP) markers. Forty-eight DNA fragments were selected based on random amplified polymorphic DNAs (RAPD) amplified across 48 individuals from each parental species, and were transformed into 44 sequence-characterized amplified region (SCAR) markers. Five SNP markers that generated species-specific alleles for each species were selected from the 28 sequenced SCARs. A multiplex single nucleotide primer extension (SNuPE) analyses of the five SNPs using 40 *A. mangium*, 40 *A. auriculiformis* and 16 *Acacia* hybrids showed high discrimination power. This diagnostic system, with high discriminatory ability, provides a highly reliable and fast method for identifying interspecific hybrids of *A. mangium* and *A. auriculiformis*.

Key words: Acacia, interspecific hybrid, PCR, RAPD, SCAR, SNP.

Introduction

Acacia mangium Willd. is native to northern Queensland in Australia; the Western Province of Papua New Guinea; and Indonesia (Irian Jaya and Maluku), and *A. auriculiformis* A. Cunn. ex Benth. is native to Cape York Peninsula, Queensland and northern areas of the Northern Territory in Australia, western and southern Papua New Guinea, and Irian Jaya and the Kei Islands in Indonesia (Turnbull, 1986). As the two species are closely related, hybrids between them can occur spontaneously or artificially (Sedgley *et al.,* 1992), and have great potential for commercial development since. Hybrids tend to exhibit improved stem straightness and roundness, and growth performance, and show intermediate wood characteristics between *A. mangium* and *A. auriculiformis*, and could be used for the development of disease-resistant lines (Pinso and Nasi, 1991).

Tree-breeding programs for interspecific hybrids rely on an accurate identification method for parental species and certification of putative hybrids to allow screening of germplasm at an early stage to ensure the delivery of the desired material and obtain the predicted genetic gains (Gros-Louis *et al.,* 2005). Many studies of hybrid identification and discrimination have been conducted; including studies of isoenzyme in pine (Korol *et al.,* 1995); RAPD markers in spruce (Perron *et al.,* 1995; Nkongolo, 1999), larch (Scheepers *et al.,* 2000) and eucalyptus (Delaporte *et al.,* 2001); simple sequence repeat (SSR) markers in poplar (Bekkaoui *et al.,* 2003; Khasa *et al.,* 2005) and *Acacia* hybrid (Ng *et al.,* 2005); cytoplasmic DNA such as chloroplast DNA (cpDNA) in eucalyptus (Mc Kinnon *et al.,* 2001); cpDNA, mitochondrial DNA (mtDNA) in larch (Achere *et al.,* 2004); and cpDNA and nuclear DNA (nDNA) in poplar (Holderegger *et al.,* 2005).

A new type of marker, single nucleotide polymorphisms (SNP) (Gupta *et al.,* 2001; Rafalski, 2002; Lee *et al.,* 2004), have attracted much attention due to their abundance in the genome and potential for use in automated high-throughput genotyping. SNPs have been used for various applications in humans (Vallone *et al.,* 2004; Lee *et al.,* 2005), animals (Heaton *et al.,* 2002; Werner *et al.,* 2004), and plants (Batley *et al.,* 2003; Varshney *et al.,* 2007). SNP markers have a number of advantages over other markers, as they have a low mutation rate and stable inheritance. Moreover, since SNP possesses a bi-allelic nature, scoring its genotype on the basis of raw data is relatively simple.

Therefore, the main objective of this study was to develop a highly reliable and accurate diagnosis system for *A. mangium*, *A. auriculiformis* and their interspecific hybrids using SNP markers.

Materials and Methods

Plant material and DNA extraction

The samples used in this study include 48 individuals of each species from a provenance trial site at The Center for Forest Biotechnology and Tree Improvement (CFBTI) in Wonogiri, Central Java, Indonesia. Details of provenance seed origins are presented in *Table 1*. Sixteen *Acacia* hybrid samples from Quy Nhon Plantation Forest Company of Vietnam Ltd. (QPFL) were also added so that the discriminability of the new diagnosis system could be confirmed. Leaf genomic DNA was isolated using a modified CTAB method (Shiraishi and Watanabe, 1995) and purified with MagneSil (Promega) following the procedure described by the supplier.

RAPD fragment screening

Sixty-four 13-mer RAPD primers were used for screening DNA fragments. PCR amplification was car-

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DOI:10.1515/sg-2011-0012
edited by Thünen Institute of Forest Genetics

Table 1. – List of *A. mangium* and *A. auriculiformis* samples used for selecting species-specific markers.

Country	*A. mangium*		*A. auriculiformis*	
	Provenance	No. of samples	Provenance	No. of samples
Papua New Guinea (PNG)	Arufi Village WP	3	Bandaber N of Bulla WP	2
	BimadebunWP	4	Bensbach WP	4
	Boite Ne Morehead WP	3	Morehead R Rouku WP	5
	Derideri E Morehead	3		
	Dimisisi WP	3		
	Gubam Ne Morehead WP	3		
	Kini WP	4		
	Wipim District WP	3		
Queensland (QLD), Australia	135K NNE Coen	3	Boggy Creek	4
	Cassowary CK-Iron Range	3	E. Normamby River	2
	Claudie R & Iron RA	4	Kennedy River	5
	Claudia River	5	Lower Poscoe River	5
	Claudie River (Ex ACEB)	2	Morehead River	4
	Pascoe River	3	Olive River	5
	Pascoe RVR Area SL 35815	2	(R) Orchard Melville Int	6
			Wenlock R & Tribs	2
			Wenlock River	4
	Total	48	Total	48

ried out in 10 µl of reaction mixture containing 10 mM Tris-HCl (pH 8.3), 10 mM KCl, 3.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.025 U/µl Platinum Taq DNA polymerase (Invitrogen), 1.5 µM of primer, and 10 ng/µl of template DNA. Amplification was performed using a thermocycler (Biometra) with an initial denaturizing process at 94°C for 1 min; 45 cycles of 94°C for 30 sec, 37°C for 30 sec, and 72°C for 90 sec; and a final cycle of 72°C for 7 min. The amplified products were verified on 1.2% agarose gel, visualized by ethidium bromide staining, and photographed using a 302-nm UV transilluminator. RAPD fragments were scored in a binary data matrix as 1 (present) or 0 (absent).

SCAR marker development

Target fragments in the RAPD screening were excised from the gel using pipette-tips. A re-amplification was carried out according to the method described above except for differences in the primer concentration (0.5 µM) and the amplification condition (30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 90 sec). The PCR product was then ligated with a plasmid vector (pGEM-T Vector System, Promega) and transformed into *E. coli* (JM109 Competent Cell, TOYOBO) according to the method recommended by the supplier. A colony PCR (HATTORI *et al.,* 1997) was amplified with the same components described above using two primers (pGE_U: 5'-TCCGGCTCGTATGTTGTGTGGA-3' and pGE_L: 5'-GTGCTGCAAGGCGATTAAGTTGG-3') for 29 cycles of 96°C for 15 sec and 70°C for 2 min followed by one cycle of 70°C for 10 min in Gene Amp 9600 (Perkin Elmer).

From 10 µl of the PCR product, 5 µl were electrophoresed in 1.2% agarose gel to confirm fragment presence. If the amplification was successful, the other 5 µl of the PCR product were then enzymatically treated with 1 U each of Shrimp Alkaline Phosphatase (SAP) and Exonuclease I (ExoI) to degrade the excess of primers and dNTPs. The reaction solution was mixed thoroughly and incubated at 37°C for 1 hour, followed by 15 minutes at 75°C to inactivate the enzymes. The sequencing reaction was performed using Big Dye Terminator v1.1/v.3.1 and -21 M13/M13 REV primer (Applied Biosystems). The reactions were purified using the CleanSEQ Sequencing Reaction Clean-up system (Agencourt) according to the supplier-recommended procedure. Sequence analysis was conducted using the ABI 3100 Genetic Analyzer (Applied Biosystems). For each successfully sequenced RAPD fragment, a pair of SCAR primers (*Table 2*) were designed based on the fragment's nucleotide sequence. SCAR amplification was conducted according to the re-amplification procedure above except for differences in the primer concentration (0.2 µM of each primer) and amplification time (30 cycles of 72°C for 60 sec in extension).

Sequencing for SCARs was carried out using four samples from each *Acacia* species according to the procedure mentioned above except for using SCAR primers (*Table 2*) as sequencing primers. The resulting sequences of each SCAR were aligned using Sequencher 4.2.2 (Gene Codes), a putative interspecies-specific SNP was selected, and an extension primer (*Table 3*) was designed based on the SNP flanking sequence.

SNuPE analysis

Amplification of multiplex SCAR was performed using the optimized primer concentration. The concentration for each SCAR primer is shown in *Table 2*. The condi-

DOI:10.1515/sg-2011-0012
edited by Thünen Institute of Forest Genetics

Table 2. – RAPD and SCAR primer sequences and SCAR primer concentrations used in a multiplex SNuPE analysis.

Marker	RAPD		SCAR	
	Primer sequence (5′ to 3′)	Fragment length (bp)	Primer sequence (5′ to 3′)	Primer conc. (μM)
AHsnp1	AACGGTGACCGTA	700	F: ACGGTGACCGTACTCCTTCGC	0.06
			R: GTGACCGTAGCCGCCGAATAC	0.06
AHsnp2	AGTCGGGTGGCGT	700	F: GTCGGGTGGCGTGGAAGGGT	0.20
			R: GTCGGGTGGCGTTCCTCGAG	0.20
AHsnp3	GGAGGAGAGGCGT	550	F: GGAGGAGAGGCGTGCCGGGG	0.12
			R: GGAGGAGAGGCGTCCAACCA	0.12
AHsnp4	GGAGGAGAGGAGC	350	F: GGAGGAGAGGAGCCAGTTGAG	0.12
			R: GGAGGAGAGGAGCACTCACACA	0.12
AHsnp5	GGAGGAGAGGAGC	250	F: GGAGGAGAGGAGCGTAGCCC	0.20
			R: GGAGGAGAGGAGCCCATTAGG	0.20

Table 3. – Extension primer sequences and concentrations for a multiplex SNuPE analysis, and bi-allele migrations in five markers.

Marker	Extension primer sequences (5′ to 3′)	Primer length (nt)	Primer concentration (μM)[a]	Allele M[b]		Allele A[b]	
				Base	Size[c]	Base	Size[c]
AHsnp1	CATGGCTTCTGCATTAC	17	1.0	A	33.2	C	33.3
AHsnp2	T_6-AGCTCGCTATATATGTT	23	1.0	C	36.2	A	35.8
AHsnp3	T_{12}-GGTTCGAGCTTGGAATC	29	0.7	G	38.5	A	39.4
AHsnp4	T_{14}-TAGCCTCCGACGTTGGC	31	0.8	T	42.6	A	40.6
AHsnp5	T_{18}-TCACTATTTCTTTCTCG	35	1.0	C	44.8	T	46.3

[a] Concentration for each extension primer used in multiplex SNuPE analysis.
[b] Alleles M and A are species-specific alleles in *A. mangium* and *A. auricaliformis*, respectively.
[c] The measured size for each primer might be slightly different in different PCR and multiplex SNuPE analysis.

tions for multiplex SCAR using the touchdown PCR (Don *et al.,* 1991) were as follows: 94°C for 30 sec and 60°C for 60 sec in the first cycle; then the annealing temperature was decreased 0.5°C every cycle from 60°C to 55°C, at which temperature 9 cycles were carried out; 20 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec.

The electrophoretic mobility of the extension products was adjusted by varying the length of the extension primer using 5' non-homologous (poly T) tails (*Table 3*). Extension reactions were carried out in 10-μl reactions containing 0.5 μl of ABI Prism SNaPshot Multiplex Ready Reaction Mix (Applied Biosystems), 1.8 μl of 5X Sequencing Buffers (Applied Biosystems), 1 μl of multiplex PCR products, and extension primers whose concentrations were adjusted as shown in *Table 3*. Extension reaction was performed in a Thermocycler (Biometra) for 25 cycles of 96°C for 10 sec, 50°C for 5 sec, and 60°C for 30 sec. One unit of SAP was added to remove the 5' phosphoryl groups that alter the migration of the unincorporated ddNTPs and thus prohibit interference. The reaction was incubated at 37°C for 1 hour followed by 15 minutes at 75°C to inactivate the enzymes.

Fluorescently labeled extension products were mixed with Hi-Di Formamide (Applied Biosystems) and LIZ-120 internal sizing standard (Applied Biosystems), and denaturized at 95°C for 5 minutes for loading onto an ABI 3100 Genetic Analyzer. Data analysis was performed using GeneMapper v 3.7 Software (Applied Biosystems).

Results

SCAR marker development

RAPD fragment screening across 48 samples for each of *A. mangium* and *A. auriculiformis* provided reproducible and reliable data for the selection of fragments for *Acacia* hybrid diagnosis. Forty-eight informative RAPD fragments were obtained (*Figure 1*), and SCAR markers were successfully designed for 44 out of the 48 fragments. Sequence analyses of newly designed SCARs were carried out using four samples each of *A. mangium*

DOI:10.1515/sg-2011-0012
edited by Thünen Institute of Forest Genetics

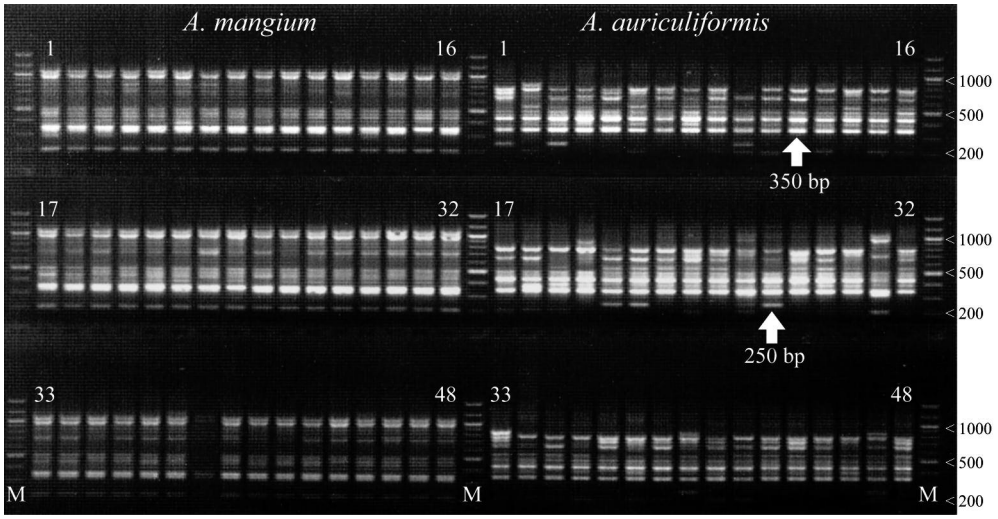

Figure 1. – Screening of RAPD fragments using 5'-GGAGGAGAGGAGC-3' primer. Two fragments at positions 250 bp and 350 bp were selected and developed into SCAR markers. M is a size marker.

and *A. auriculiformis*. Analysis of the SCAR markers showed that 43 SCARs were successfully amplified and only one fragment could not be amplified. Twenty-one SCARs produced monomorphic fragments in both *A. mangium* and *A. auriculiformis*, 14 SCARs had no fragment in *A. mangium* with monomorphic/polymorphic fragments in *A. auriculiformis*, and eight highlighted polymorphism in one or both *Acacia* species.

SNP detection

Twenty-eight SCARs, including 21 monomorphic SCARs, were further sequenced for four samples of each Acacia species. SNPs in the form of heterozygotes, point mutations and insertion/deletions (indel) at a specific base position were detected. Twenty-five out of 28 SCARs produced informative sequence data. Two SCARs did not detect any variation, while one failed to produce good sequence data. Fifteen SCARs that were considered to have useful polymorphism for hybrid diagnosis were further amplified to verify the presence of the fragment in all tested samples (48 in each *Acacia* species) and also to check PCR fragment quality. Five SCARs (AHsnp 1-5 in *Tables 2*) were finally selected and developed into extension primers (*Figure 2* and *Table 3*).

SNuPE analysis

To avoid overlapping among the five markers in multiplex analyses, the extension primer length of each marker was adjusted by adding Poly-thymidine (Poly-T) tail to the 5' end following the procedure described by Applied Biosystems (*Table 3*). The signal intensity of the target allele for each primer in the multiplex analysis was optimized. PCR primer concentration of the marker that exhibited high signal intensity was reduced, where-

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        110                                                         170
AM01    ACCATCCACGGCGTAGCCTCCGACGTTGGCTGCGTGATTCTTTTGGCACTCTCCATATCTC
AM02    ACCATCCACGGCGTAGCCTCCGACGTTGGCTGCGTGATTCTTTTGGCACTCTCCATATCTC
AM03    ACCATCCACGGCGTAGCCTCCGACGTTGGCTGCGTGATTCTTTTGGCACTCTCCATATCTC
AM04    ACCATCCACGGCGTAGCCTCCGACGTTGGCTGCGTGATTCTTTTGGCACTCTCCATATCTC
AR01    ACCATCCACGGCGTAGCCTCCGACGTTGGCAGCGTGATTCTTTTGGCACTCTCCATATCTC
AR02    ACCATCCACGGCGTAGCCTCCGACGTTGGCAGCGTGATTCTTTTGGCACTCTCCATATCTC
AR03    ACCATCCACGGCGTAGCCTCCGACGTTGGCAGCGTGATTCTTTTGGCACTCTCCATATCTC
AR04    ACCATCCACGGCGTAGCCTCCGACGTTGGCAGCGTGATTCTTTTGGCACTCTCCATATCTC

        33                                                          94
AM01    GTGTGAGAAGTATTTTGGAGAATCCCTTCTTATCACTATTTCTTTCTCGCCTCTCTCTCTC
AM02    GTGTGAGAAGTATTTTGGAGAATCCCTTCTTATCACTATTTCTTTCTCGCCTCTCTCTCTC
AM03    GTGTGAGAAGTATTTTGGAGAATCCCTTCTTATCACTATTTCTTTCTCGCCTCTCTCTCTC
AM04    GTGTGAGAAGTATTTTGGAGAATCCCTTCTTATCACTATTTCTTTCTCGCCTCTCTCTCTC
AR01    GTGTGAGAAGTATTTTGGAGAATCCCTTCTTATCACTATTTCTTTCTCGTCTCTCTCTCTC
AR02    GTGTGAGAAGTATTTTGGAGAATCCCTTCTTATCACTATTTCTTTCTCGTCTCTCTCTCTC
AR03    GTGTGAGAAGTATTTTGGAGAATCCCTTCTTATCACTATTTCTTTCTCGTCTCTCTCTCTC
AR04    GTGTGAGAAGTATTTTGGAGAATCCCTTCTTATCACTATTTCTTTCTCGTCTCTCTCTCTC
```

Figure 2. – Partial sequence of *A. mangium* (AM) and *A. auriculiformis* (AR) genome using AHsnp4 marker (upper) and AHsnp5 marker (lower). Bold letters represent the target SNP. The underlined sequences represent extension primer sequences.

DOI:10.1515/sg-2011-0012
edited by Thünen Institute of Forest Genetics

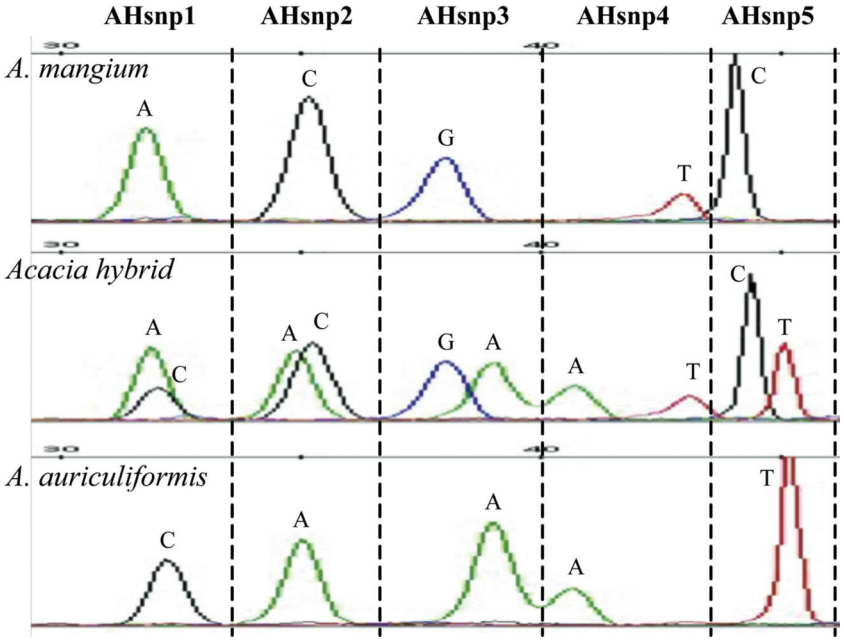

Figure 3. – Signal intensity of *A. mangium*, *Acacia* hybrid and *A. auriculiformis* using five markers (AHsnp1, AHsnp2, AHsnp3, AHsnp4, and AHsnp5) in a multiplex SNuPE analysis. The letter on the top of each peak refers to the SNP allele.

Table 4. – Bi-allele frequencies of five markers in an *A. mangium*, *A. auriculiformis* and *Acacia hybrid*.

Marker	*A. mangium* (40)[a]		*A. auriculiformis* (40)[a]		*Acacia* hybrid (16)[a]	
	Allele M[b]	Allele A[b]	Allele M[b]	Allele A[b]	Allele M[b]	Allele A[b]
AHsnp1	1.00	0.00	0.00	1.00	1.00	1.00
AHsnp2	1.00	0.00	0.00	0.95	1.00	1.00
AHsnp3	1.00	0.08	0.00	1.00	1.00	1.00
AHsnp4	1.00	0.00	0.00	1.00	1.00	1.00
AHsnp5	1.00	0.00	0.00	1.00	1.00	0.94

[a] Number in parenthesis represents number of sample analyzed.
[b] Alleles M and A are species-specific alleles in *A. mangium* and *A. auricaliformis*, respectively.

as other primers were kept at a standard concentration (0.2 µM). After the optimized PCR primer concentrations were obtained (*Table 2*), further optimization to balance the extension primer concentrations was conducted (*Table 3*). The process was repeated several times until a balanced signal was obtained across all markers (*Figure 3*).

Discrimination power of the multiplex SNuPE analysis

The discrimination power of the multiplex SNuPE for the diagnosis of interspecific *Acacia* hybrids and their parental species was tested using 40 *A. mangium*, 40 *A. auriculiformis* and 16 *Acacia* hybrid samples. AHsnp1 and AHsnp4 markers were able to detect *A. mangium*-specific alleles and *A. auriculiformis*-specific alleles, respectively. For AHsnp3, allele A specific to *A. auriculiformis* was also detected in three *A. mangium* samples (8%). For AHsnp2, allele A specific to *A. auriculiformis* could not be detected in two *A. auriculiformis* samples. For AHsnp5, *A. auriculiformis*-specific allele A could not be obtained in a hybrid sample (*Table 4*).

Discussion

RAPD markers (William *et al.,* 1990) and SCAR markers (Paran and Michelmore, 1993) were employed to obtain useful fragments for hybrid diagnosis between *Acacia mangium* and *A. auriculiformis*. RAPD markers have been a commonly used method for hybrid identification; however, they are difficult to reproduce, and are sensitive to minor changes in reaction conditions (Jones *et al.,* 1997; Mc Gregor *et al.,* 2000). The reliability of fragments screening was improved by developing candidate RAPD fragments into SCAR markers. SCARs have several advantages over RAPD markers, as they can target single defined loci, are less sensitive to reaction

DOI:10.1515/sg-2011-0012
edited by Thünen Institute of Forest Genetics

conditions, and scoring results is more straightforward (PARAN and MICHELMORE, 1993; WENG *et al.,* 1998; BAUTISTA *et al.,* 2002).

Identification of interspecific hybrids between *A. mangium* and *A. auriculiformis* using only two SCAR markers (HUANG *et al.,* 2005) had been previously reported. These SCARs have been used as a means for identifying hybrids. However, in order to achieve more reliable diagnosis, more species-specific markers should be used. Fifteen polymorphic microsatellite markers have also been developed for *Acacia* hybrid (NG *et al.,* 2005). However, using such marker to routinely screen for hybrids might be problematic since the microsatellite markers are polymorphic in the parental species and possess intraspecific variation.

SNP-based markers offer many advantages over microsatellite markers. Namely, they are direct markers (BATLEY *et al.,* 2003), have low mutation rates (VIGNAL *et al.,* 2002; BATLEY *et al.,* 2003; BRUMFIELD *et al.,* 2003; AITKEN *et al.,* 2004) and are more stable than other marker systems (GUPTA *et al.,* 2001). SNPs also offer a great opportunity for unbiased sampling of loci (BRUMFIELD *et al.,* 2003) and simple allele nomenclature (VIGNAL *et al.,* 2002). Therefore, the SNP marker system not only offers lower cost (an estimated five to ten times lower than for microsatellites), higher repeatability and higher accuracy, but it also offers increased opportunities to develop germplasm databases that are meaningful across different organizations (JONES *et al.,* 2007).

The high discrimination power of the new markers was confirmed using 96 *Acacias* and hybrids. In AHsnp1 and AHsnp4, 100% of *A. mangium*-specific alleles and 100% of *A. auriculiformis*-specific alleles were detected in a multiplex SNuPE analysis. However, an *A. auriculiformis*-specific allele in AHsnp3, was also detected at low levels in *A. mangium*, and *A. auriculiformis*-specific alleles in AHsnp2 and AHsnp5 were not detected in a few samples of *A. auriculiformis* nor in hybrid (*Table 4*). This suggests that the *A. auriculiformis* infrequently holds null alleles in addition to the species-specific alleles for these two markers.

The ability of SNP to be applied as species-specific markers found in this research is consistent with other studies. A study of soybeans for cultivar identification showed that a panel of 23 SNPs could uniquely distinguish 2,200 soybean cultivars (YOON *et al.,* 2007). Another study on spruce demonstrated that two SNPs (one chloroplast DNA and one nuclear DNA) can distinguish black spruce from red and white spruces at a rate of 96–100% and that 5 SNPs (four chloroplast and on nuclear) can distinguish white spruce from red and black spruces at a rate of 100% (GERMANO and KLEIN, 1999). The infrequent presence of a specific allele in another species was also discovered in this research. The fact that the *A. auriculiformis*-specific allele (AHsnp3 allele A in *Table 4*) was present in *A. mangium* samples suggested the possibility that a common allele is shared between *A. mangium* and *A. auriculiformis*. However, the existence of null alleles (AHsnp2 allele A and AHsnp5 allele A in *Table 4*) in *A. auriculiformis* samples and *Acacia* hybrids might be the result of mutations, either insertion/deletion (indel) or base substitution, in the priming site of the extension primer used for SNuPE analysis.

The multiplexing of SNPs has been used extensively in work on humans (BRANDSTATTER *et al.,* 2003; VALLONE *et al.,* 2004; VALLONE and BUTLER, 2004; VALLONE *et al.,* 2006) and plants (BATLEY *et al.,* 2003; MOHRING *et al.,* 2004). This paper describes a multiplex SNP analysis designed for the diagnosis of interspecific hybrids between *A. mangium* and *A. auriculiformis* and their parental species. The system has a high capacity to discriminate five *A. mangium*-specific alleles and five *A. auriculiformis*-specific alleles. Therefore, this multiplex system provides reliable, accurate and fast identification of the species-specific alleles in *A. mangium* and *A. auriculiformis.*

In conclusion, we have developed a multiplex SNuPE analysis consisting of five markers that can detect species-specific alleles in *A. mangium* and *A. auriculiformis*. The markers have high reliability for the accurate discrimination of the interspecific hybrid between *A. mangium* and *A. auriculiformis*, as well as their parental species. Various applications can be performed using this system, including; the confirmation of hybrid identity from spontaneous or artificial hybridization, the certification of commercial hybrid plantlets for afforestation, and the selection of plus hybrids from a plantation/nursery. Moreover, using this new diagnosis system, efficiency and clarification of the hybrid product can be obtained in hybrid cultivar development using a bi-species seed orchard of *A. mangium* and *A. auriculiformis* species.

Acknowledgements

We are grateful to Drs. AYPBC WIDYATMOKO and ARIF NIRSATMANTO of The Center for Forest Biotechnology and Tree Improvement, Forestry Research and Development Agency, Indonesia who provided the *A. mangium* and *A. auriculiformis* materials, and to Dr. KAZUYA ITO, Oji Paper Co., Ltd. for offering the hybrid materials. The author also thanks Drs. ANTO RIMBAWANTO and JEANINE PFEIFFER for their valuable comments on the manuscript.

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DOI:10.1515/sg-2011-0012
edited by Thünen Institute of Forest Genetics

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DOI:10.1515/sg-2011-0012
edited by Thünen Institute of Forest Genetics

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Short Note: Seven Genomic SSRs Revealed in *Eucalyptus* by Re-sequencing of DNA Sequences from GenBank

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(Received 28th January 2010)

Abstract

Seven genomic SSR markers of *Eucalyptus* were developed from DNA sequences of *E. grandis* deposited in GenBank. Their repeat motifs were revealed by re-sequencing with an individual tree of *E. urophylla* or *E. tereticornis*, and five out of the seven markers turned out to be heterozygous within the specific tree sequenced. The sequence identity ranged from 75.06% to 96.66%, with an average of 87.31%. These markers could be valuable in genetics studies in *Eucalyptus*. This report demonstrates the advantages of re-sequencing in developing SSR markers from publicly accessible databases.

Key words: Simple sequence repeats (SSRs), microsatellites, molecular markers, *Eucalyptus*, re-sequencing.

Simple sequence repeats (SSRs), as known as microsatellites, are useful markers for a wide spectrum of biological applications (POWELL et al., 1996; GUPTA and VARSHNEY, 2000). To date, a large number of SSR markers have been produced for a number of plant species, e.g. more than 5000 SSRs available for sorghum (*Sorghum bicolor*) (YONEMARU et al., 2009). In the woody genus *Eucalyptus* (family Myrtaceae), however, only 367 genomic SSRs (as reviewed in BRONDANI et al., 2006) and 68 EST-SSRs (FARIA et al., 2010, 2011; ZHOU et al.,

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DOI:10.1515/sg-2011-0012
edited by Thünen Institute of Forest Genetics