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SHORT COMMUNICATION

Genetic Diversity of *Acacia mangium* Seed Orchard in Wonogiri Indonesia Using Microsatellite Markers

VIVI YUSKIANTI[1*], KEIYA ISODA[2‡]

[1]Center for Forest Biotechnology and Tree Improvement, Jalan Palagan Tentara Pelajar Km 15, Purwobinangun, Pakem, Sleman, Yogyakarta 55582, Indonesia
[2]Forest Tree Improvement Project Phase II, JICA, Jalan Palagan Tentara Pelajar Km 15, Purwobinangun, Pakem, Sleman, Yogyakarta 55582, Indonesia

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Genetic diversity is important in tree improvement programs. To evaluate levels of genetic diversity of first generation *Acacia mangium* seedling seed orchard in Wonogiri, Central Java, Indonesia, three populations from each region of Papua New Guinea (PNG) and Queensland, Australia (QLD) were selected and analyzed using 25 microsatellite markers. Statistical analysis showed that PNG populations have higher number of detected alleles and level of genetic diversity than QLD populations. This study provides a basic information about the genetic background of the populations used in the development of an *A. mangium* seed orchard in Indonesia.

Key words: *Acacia mangium*, genetic diversity, microsatellite markers, seedling seed orchard

INTRODUCTION

Seed orchards are cultivated for their abundant production of easily harvestable seeds from superior trees and are established by setting out clones or seedling progeny of trees selected for desired characteristics (Millar *et al*. 2008). The objective of a seed orchard is to produce maximum amounts of seed with as much genetic improvements, as quickly, and as efficiently as possible (Zobel & Talbert 1984). Knowledge on the genetic diversity of the populations used in the development of the first-generation seed orchard is important. Genetic diversity is of prime importance for species persistence (Geburek 1997) and also plays a critical role in the survival of populations that are challenged by changing environments (Bawa & Dayadan 1998).

Studies on the level of genetic diversity in *Acacia mangium* found that PNG populations (Pongaki, Makapa, Muting, and Lake Murray) have the highest genetic diversity, followed by Australian population, but declining from north to south; Townsville and Daintree populations had approximately half the genetic variation of Cape York populations, e.g. Claudie River and Captain Billy Road (Butcher *et al*. 1998). Outcrossing rates in natural populations ranged from complete selfing in outlying populations with low genetic diversity to complete outcrossing in the more variable populations (Butcher *et al*. 2004).

Microsatellite markers have been used for genetic diversity studies in many species (Burle *et al*. 2010; Hardesty *et al*. 2010; Bloomfield *et al*. 2011). The markers are higly polymorphic, codominant, and have more informations (Rajora & Rahman 2003). This study was conducted to analyze the level of genetic diversity in the first-generation seedling seed orchard (SSO) of *A. mangium* at Wonogiri, Center Java, Indonesia.

MATERIALS AND METHODS

Seedling Details on Seed Orchard. The first-generation SSO of *A. mangium* at Wonogiri, Center of Java, Indonesia was established in 1994 in cooperation with the Japan International Cooperation Agency (JICA). The site is at 7^0 32' South latitude and 110^0 41' East longitude. The elevation, slope and soil type are 141 m, 10%, and a vertisol, respectively. The average annual rainfall, maximum, and minimum temperatures are 1878 mm, 38.2 ^{o}C, and 21.2 ^{o}C, respectively. The orchard has 144 families of *A. mangium* [69 representing 8 provenances from Papua New Guinea (PNG) and 75 representing 8 provenances from Queensland, Australia (QLD)], each in 4-tree plots, with 7 replications; the spacing is 4 m between and 2 m within rows (Nirsatmanto & Hashimoto 1995).

Sample Collection. Three provenances from PNG (Kini WP, Gubam Ne Morehead WP, and Wipim Discrict WP) and three provenances from QLD (Claudie River-135K Ne Coen, Cassowary CK-Iron Range and Pascoe River) were chosen for this study. Ten trees were chosen from each

‡Current address: Kansai Regional Breeding Office, Forest Tree Breeding Center, Forestry and Forest Products Research Institute, 1043 Uetsukinaka, Syouohcho, Katutagun, Okayama 709-4335, Japan

*Corresponding author. Phone: +62-274-895954, Fax: +62-274-896080, E-mail: vivi_yuskianti@yahoo.com

provenance, except Claudie River-135 provenance from QLD (14 trees) to reach a total of 64 trees. Two to three leaves were collected from each tree and kept in an envelope with silica gel and stored in a refrigerator due to DNA extraction.

DNA Extraction. Genomic DNA was isolated using a modified protocol of the CTAB method (Shiraishi & Watanabe 1995).

DNA Analysis. Twenty-five microsatellite markers (Butcher *et al*. 2000) were used for genotyping the trees. PCR analysis was carried out in a 10 µl reaction containing 1 x PCR buffer, 1.5 to 3.0 mM $MgCl_2$, 0 or 1% formamide, 200 µM each dNTP, 0.5 µM each Primer, 0.5 unit *AmpliTaq Gold* DNA polymerase (PE Applied Biosystems), and 25 ng template DNA. Optimized $MgCl_2$ concentration for each marker was used to obtain the minimum or maximum single nucleotide addition caused by the *Taq* DNA polymerase (Table 1).

PCR DNA amplification was conducted at 94 °C for 10 minutes, 35 cycles at 94 °C for 30 seconds, 50-60 °C for 30 seconds, 72 °C for 60 seconds, followed by 1 minute at 72 °C for the extension step in a GeneAmp PCR System 9700 (PE Applied Biosystems). Touch down PCR was used for PCR analysis. The annealing temperature for the initial cycle was 65 °C, and the temperature was decreased by 1 °C/cycle for the following 9 cycles. After the initial 10 cycles, the annealing temperature was fixed at 55 °C. PCR products were electrophoresed using an ABI 310 Genetic Analyzer (PE Applied Biosystems). The lengths of the PCR products were determined using GeneScan and Genotyper software (PE Applied Biosystems) and interpreted for the alleles.

Data Analysis. The genetic parameters, such as the number of detected alleles (A), allelic richness ($A_{(63)}$), observed heterozygosity (H_o), expected heterozygosity (H_e) and coefficient of inbreeding (F_{is}), were analyzed using FSTAT software version 2.9.3.2. The genetic differentiation among populations was calculated using F_{st} values. The significance of F_{st} values was tested using randomizing multilocus genotypes between the two populations with standard Bonferroni corrections.

RESULTS

Polymorphisms of Microsatellites Markers. Analysis using FSTAT statistics showed that the number of detected alleles (A) for all markers varied from 2 to 25; the mean was 8.48 (Table 1). The observed (H_o) and expected (H_e) heterozygosities ranged from 0.047 to 0.781 and 0.046 to 0.896 (Table 1). Furthermore, trinucleotide microsatellites (Am 522 and 770) produced lower numbers of alleles (A = 3) than dinucleotide microsatellites (A = 8.95) (Table 1).

Genetic Diversities of All Populations in the Orchard. In general, PNG provenances had higher number of detected alleles (A), allelic richness ($A_{(63)}$), H_o, and H_e than QLD provenances. For PNG provenances, the number of detected alleles ranged from 5.72 to 5.28; while for QLD provenances 3.52 to 3.36 (Table 2). Levels of inbreeding

Table 1. Genetic diversity of the 25 microsatellite markers in 64 tree samples from PNG and QLD populations

Marker	$MgCl_2$ concentration	N	A	$A_{(63)}$	H_o	H_e	F_{is}
Dinucleotide microsatellites							
Am012	3.0 mM	64	8	7.984	0.703	0.784	0.103
Am014	2.5 mM	64	22	22.000	0.683	0.896	0.238
Am041	3.0 mM + 1% formamide	64	25	24.828	0.719	0.865	0.169*
Am136	3.0 mM + 1% formamide	64	9	8.984	0.781	0.798	0.021
Am173	3.0 mM	64	8	8.000	0.578	0.629	0.081
Am326	2.5 mM	64	15	14.922	0.547	0.810	0.325
Am341	1.5 mM	64	5	4.984	0.375	0.457	0.179
Am352	3.0 mM	64	6	5.969	0.157	0.702	0.777
Am384	3.0 mM	64	3	2.984	0.344	0.390	0.119
Am387	1.5 mM	64	10	9.969	0.703	0.773	0.090
Am389	3.0 mM	64	12	11.984	0.703	0.808	0.130
Am391	3.0 mM	64	5	4.953	0.140	0.134	-0.047
Am400	3.0 mM	64	2	2.000	0.375	0.473	0.208
Am424	3.0 mM	64	4	4.000	0.406	0.347	-0.169
Am429	1.5 mM	64	9	8.984	0.672	0.807	0.167*
Am435	2.5 mM	64	10	9.937	0.781	0.735	-0.063
Am436	2.5 mM	64	8	8.000	0.547	0.739	0.260
Am460	1.5 mM	64	5	5.000	0.578	0.743	0.222*
Am463	3.0 mM	64	4	4.000	0.562	0.590	0.047
Am465	3.0 mM + 1% formamide	64	15	14.953	0.703	0.778	0.096
Am477	3.0 mM	64	4	3.984	0.188	0.227	0.174
Am484	3.0 mM	64	11	10.969	0.454	0.834	0.456
Am503	1.5 mM	64	6	5.984	0.468	0.693	0.324
Mean		64	8.956	8.929	0.529	0.653	0.169
Trinucleotide microsatellites							
Am522	3.0 mM	64	3	2.984	0.047	0.046	-0.011
Am770	3.0 mM	64	3	2.984	0.344	0.513	0.330*
Mean		64	3	2.984	0.196	0.279	0.159
Mean (all markers)	64	64	8.48	8.454	0.502	0.623	0.169

*significant *Fis* with 500 randomizations at the P < 0.05 level. N: tree sample size, A: number of detected alleles, $A_{(63)}$: Allelic richness, H_o: observed heterozygosity, H_e: expected heterozygosity, F_{is}: fixation index, PNG: Papua New Guinea, QLD: Queensland Australia.

(F_{st})in QLD (0.125) were higher than that of in PNG populations (0.078) (Table 2). Analysis on the frequency of alleles in the PNG population and the QLD population showed that the PNG population had higher frequency of alleles than the QLD population (Data not shown).

DISCUSSION

Twenty five microsatellite markers used in this study provided data, with high reliability and accuracy, for the analysis of the genetic diversity level of the material used in the development of the first generation *A. mangium* SSO at Wonogiri, Central Java, Indonesia.

This study found that the number of detected alleles (*A*) varied from 2 to 25; the mean was 8.48 (Table 1). This value was higher than other microsatellite studies found for *Pinus merkusii* (*A*=3 to 8 in parental and 3 to 9 for offspring populations; average = 4.8) (Nurtjahjaningsih *et al*. 2007) and Kilakarsal sheep (*A* = 3 to 13; average = 7.6) (Radha *et al*. 2011), but lower than in *Eucalyptus dunnii* (*A* = 10 to 14) (Poltri *et al*. 2003) and *Acacia saligna* subsp. *saligna* (*A* = 6 to 16; average = 9.4) (Millar *et al*. 2008).

Trinucleotide microsatellites (Am 522 and 770) produced lower numbers of alleles (*A* = 3) than dinucleotide microsatellites (*A* = 8.95) (Table 1). This higher variation of dinucleotide microsatellite markers means that they are more polymorphic than trinucleotide microsatellite markers.

Genetic Diversities of All Populations in the Orchard. PNG populations had higher genetic diversity (H_e=0.652), number of detected alleles, and allelic richness than QLD populations (H_e=0.495) (Table 2). Further analysis on the allele frequencies of each population supported the results. Allele frequency analysis showed that more than 90% of alleles existed in the PNG population, but only 52.8% existed in the QLD population (Data not shown). This result was in accordance with other studies. Butcher *et al*. (1998) found the highest levels of genetic variation (allelic diversity, number of polymorphic loci and *H*) in the New Guinea natural populations of *A. mangium*. Furthermore, SSO established from the southern-most Australian populations had half the allelic richness and 30% less *H* than SSO established from PNG populations (Butcher *et al*. 2004).

The total level of genetic diversity in all populations was high (H_e=0.623) (Table 2). This level of genetic diversity was comparable to that found in other microsatellite studies in planted stands. For example, the genetic diversity in a planted stand of *A. saligna* subsp. *saligna* was 0.556 (Millar *et al*. 2008), in parental and offspring populations of *Pinus merkusii* seed orchards, the genetic diversities were 0.489 and 0.545, respectively (Nurtjahjaningsih *et al*. 2007), and at an experimental site of *Dicorynia guianensis* at Paracou it was 0.629 (Latouche-Halle *et al*. 2004). These values are lower than the levels of genetic diversity found in conservation areas and in natural populations. For example, the genetic diversities in conservation areas of *Shorea parvifolia* and *S. leprosula* were 0.664 and 0.797, respectively (Tani *et al*. 2009); in a small population of *Pinus sylvestris* L. it was 0.843-0.927 (Robledo-Arnuncio & Gil 2005).

Levels of inbreeding in QLD populations (0.125) were higher than in PNG populations (0.078) (Table 2). Inbreeding is used to describe various related phenomena that all refer to situations in which mating occurs among relatives and leads to an increase in homozygosity (Keller & Waller 2002). Inbreeding may cause lower levels of genetic diversity and negative effects on tree performance. At Subanjeriji in Sumatra, the poor performance of the Daintree QLD population might be caused by a very high level of inbreeding (70%); in contrast, there was no evidence of inbreeding in high performing populations from New Guinea and Aru (Butcher *et al*. 1999). In Vietnam, progeny from two orchards based primarily on PNG provenances with high levels of outcrossing displayed the best growth (mean heights of 3.1 m at 18 months), while another orchard based on Queensland provenances, with 51% selfed progeny, displayed the poorest growth (mean heights of 2.0 and 2.2 m repectively) (Harwood *et al*. 2004).

The genetic differentiation among populations (F_{st}) showed that all populations have moderate levels of genetic differentiation (the value of F_{st} was 0.105 with 0.105-0.161 of 95% confidence interval; data not shown). The value was lower than previously reported in *A. mangium*, which was 0.331 in RFLP study (Butcher *et al*. 1998), and also different from the conclusion of Butcher *et al*. (2004) which stated that *A. mangium* had high genetic differentiations among populations in different geographic regions. This condition might be caused by the differences in the material and DNA markers used for analysis.

Table 2. Genetic diversity of all populations, PNG, and QLD populations and each of the provenances in 25 microsatellite markers

	N	*A*	$A_{(63/29/9)}$	H_o	H_e	F_{is}
All	64	8.48	8.454	0.518	0.623	0.169
Papua New Guinea (PNG)	30	7.92	7.859	0.601	0.652	0.078
Queensland, Australia (QLD)	34	4.48	4.347	0.433	0.495	0.125
PNG Kini WP, PNG	10	5.28	5.109	0.592	0.622	0.048
Gubam Ne Morehead WP	10	4.60	4.492	0.607	0.615	0.013
Wipim District WP	10	5.72	5.478	0.592	0.648	0.087
QLD Claudie River-135K Ne Coen	14	3.52	3.211	0.434	0.512	0.152
Cassowary CK-Iron Range	10	3.36	3.268	0.392	0.469	0.165
Pascoe River	10	3.32	3.225	0.424	0.465	0.089

N: sample size, *A*: mean number of detected alleles, $A_{(63/29/9)}$: mean allelic richness (63 for all materials, 29 for PNG and QLD population, and 9 for each provenance), H_o: mean observed heterozygosity, H_e: mean expected heterozygosity, F_{is}: mean fixation index.

This study provides basic information about the genetic background of the populations used in the development of the first-generation SSO of *A. mangium* at Wonogiri, Indonesia. Its genetic diversity will determine the capability of this orchard in selecting second-generation progeny and its capacity to contribute to tree improvement. The identification of rare and common alleles will dictate strategy for the development of the next generation orchard. While the selections must consider growth performance, the choices made must also maintain high levels of genetic diversity and conserve rare alleles.

In conclusion, this study has demonstrated how 25 microsatellite markers can capture the genetic diversity in a first-generation SSO in Wonogiri, Central Java Indonesia. The result of this study supported previous studies that state that genetic diversity in PNG populations was higher than in QLD populations. Additional studies of the mating system, pollinator behaviour, flowering time, and the effectiveness of recurrent selection in open pollinated SSOs are also required to further the development of effective management strategies for maximizing genetic gains in breeding programs.

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