PARENT IDENTIFICATION IN A MULTI LOCATION TRIAL SEED ORCHARD OF *Acacia mangium* USING MICROSATELLITE MARKERS

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PARENT IDENTIFICATION IN A MULTI LOCATION TRIAL SEED ORCHARD OF *Acacia mangium* USING MICROSATELLITE MARKERS. Variation of parent gametes' contribution might affect the growth patterns among offspring produced from seed orchards. This paper studies the mating system statuses and to identify parent trees that produce good growth performance of offspring in seedling seed orchard of *A. mangium*. The study was conducted in two seed orchards, i.e. a first generation seedling seed orchard (F1 SSO) of *A. mangium,* used as the parent population hereafter and a multi location trial (MLT) as an offspring population. Based on 10 microsatellite markers, mating system in the F1 SSO was under panmictic equilibrium condition. The good growth trees in the MLT originated from various parent genes contribution in the F1 SSO. Several behaviors were observed: a). the best trees in MLT dominantly originated from single pair trees, between maternal and paternal trees, in F1 SSO; b). a maternal tree was pollinated by two paternal trees; c). a paternal tree pollinated several maternal trees. Flowering synchronization and genetic compatibility among trees might be responsible for these mating system patterns. In order to maximize seeds production, studies on male and female flowers characteristic should be employed to assess flowering synchronization among individual trees in the seed orchard.

Keywords: Parent identification, seedling seed orchard, multi-location trial, microsatellite

IDENTIFIKASI TETUA DI KEBUN BENIH UJI MULTI-LOKASI Acacia mangium *MENGGUNAKAN PENANDA MIKROSATELIT. Varisi kontribusi gamet pasangan tetua di sebuah kebun benih berakibat pada karakter pertumbuhan anakan yang dihasilkan. Tulisan ini mempelajari kepastian status sistem perkawinan dan untuk mengidentifikasi pasangan tetua yang menghasilakan tampilan pertumbuhan yang bagus pada anakan yang dihasilkan dari kebun benih semai jenis Acacia mangium. Penelitian ini dilakukan di dua kebun benih yaitu kebun benih semai generasi pertama (F1 SSO)* A. mangium*, sebagai populasi tetua, dan kebun benih uji multi-lokasi (MLT), sebagai populasi anakan. Berdasarkan sepuluh penanda mikrosatelit, sistem perkawinan di F1 SSO menunjukkan kondisi acak dan seimbang. Pohon dengan pertumbuhan bagus di MLT berasal dari variasi kontribusi gamet tetua di F1 SSO. Beberapa variasi tersebut yaitu a). pohon terbaik di MLT didominasi oleh perkawinan sepasang tetua jantan dan betina di F1 SSO; b). satu pohon tetua betina diserbuki oleh dua pohon tetua jantan; c). satu pohon tetua jantan menyerbuki beberapa pohon tetua jantan. Pembungaan serempak dan kecocokan genetik antar pohon menyebabkan terjadinya variasi sistem perkawinan tersebut. Untuk memaksimalkan produksi benih, studi mengenai karakteristik pembungaan jantan dan betina harus dilakukan untuk mengetahui keserempakan pembungaan di kebun benih.*

Kata kunci: Identifikasi tetua, kebun benih semai, uji multi-lokasi, mikrosatelit

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I. INTRODUCTION

Acacia mangium is native to Northeastern Queensland (Australia), Western Province of Papua New Guinea (PNG) and Moluccas (Aru Islands, Seram, the Sula Islands) and Irian Jaya (Sidei) of Indonesia (Orwa, Mutua, Kindt, Jamnadass, & Anthony, 2009; Saro, Robledo-Arnuncio, González-Pérez, & Sosa, 2014). It has high economical value for pulp production. This species is dominantly outcrossing with insect pollinators, especially bees and small birds which disperse the seeds (P. Butcher, Harwood, & Quang, 2004; Orwa et al., 2009). In Indonesia, demand for high quality seeds of A. mangium has been increasing, due for use as commercial plantation materials. For this reason, since 1990s a breeding strategy for the species has been established through series of progeny trials to secure production of quality and quantity seeds (Nirsatmanto, Leksono, Kurinobu, & Shiraishi, 2004). The progeny trials had been converted to seed orchards to meet demand of genetically improved seeds for commercial plantations and genetic materials for sequential generations in breeding strategies. Moreover, to test genetic gain and provide more quantity of improved seeds, a multi-location trial had been established, using seeds from the SSO.

To assure the production of high quality seeds from seed orchards, mating system should be under random mating. However, the ideal condition has rarely occurred. High selfing rates and contamination rates from unimproved stand, uneven or unbalanced mating system and variation of parental gametes' contribution have been reported as major problems in reducing quality seeds produced from seed orchard (Dering & Chybicki, 2012; El-Kassaby, Ritland, Fashler, & Devitt, 1988; Moriguchi, Taira, Tani, & Tsumura, 2004; Saro et al., 2014). Various factors influence mating system and pollen contribution mechanism including genetic factors such as flowering phenology synchronization that influence the compatibility between pollen-stigma genes interaction (Larson, Hume, Andres, & Harrison, 2012), mate choices (Maroja, Andres, Walters, & Harrison, 2009) and environmental factors, such as trees density and pollinators that affected to population size (Dow & Ashley, 1998). Many studies reported that one of the reasons in the unbalanced mating system is caused by seed orchards which are encompassed by many provenances which have differences in flowering phenology characteristics (Hansen & Kjaer, 2006).

In case of tree improvement strategy in *A. mangium* in Indonesia, the progeny trial had been converted to seedling seed orchards (called as F1 SSO hereafter). High quality seeds then could be expected from these orchards for plantation programs and genetic materials in sequential strategies including multi location trial seed orchard (called MLT, hereafter). Mating statuses in the SSO have not been assessed yet. Moreover, based on growth performances (DBH x height), selected plus trees in the F1 SSO seem to be mostly uniform with good performances. In contrast, growth performances were more variable among plus trees in MLT, with parent trees originating from the SSO. Identification of parent gametes in F1 SSO that produce good performances in the MLT might be useful to increase efficiency in sequential tree improvement strategies, especially when a clonal seed orchard is going to be established in the near future. To increase production of seeds, the CSO should be composed by mother trees that have value of general combining ability (GCA). Recently, estimation of GCA value could be approached by phenotypic observation in full-sib population, but it is difficult to identify those parents which contributed to the mating system.

Parental analysis using DNA markers provides information accurately on identification of parent gametes contribution. Recent advances in DNA markers, microsatellite or simple sequence repeat (SSR) allow genetic diversity observation in tree species. Microsatellites have 2-6 nucleotide repeat tandem and highly polymorphic allele. Microsatellites enable to detect heterozygote alleles due to co-dominant

markers and show bi-parentally inherited markers. The power of microsatellite markers had been proven to identify parents within hybridization zones of *Fraxinus* (Thomasset et al., 2014).

The study aimed to analyze mating system and to identify parent trees that may produce good growth performance offspring in seedling seed orchard of *A. mangium*.

II. MATERIAL AND METHOD

A. Description of Seed Orchards

This study was conducted in two seedling seed orchards (SSOs) of *A. mangium* i.e. a first generation seedling seed orchard (F1 SSO) and a multi location trial seed orchard (MLT). The F1 SSO was used as a parent population and MLT was used as offspring population. The F1 SSO was established in 1994 located in Wonogiri (Central Java). The SSO consisted of 144 families originating from two provenances i.e. Queensland and Papua New Guinea (PNG), that is designed as a single population which consisted of 4 sub-line systems i.e. sub line A and C from Australia; sub-line B and D from PNG. Each family were planted with 4 trees replications and they were replicated in 7 blocks. Initial spacing between trees was 4 x 2 m. The total number of trees was 4,032 trees covering an area of 3.53 Ha. The last selective thinning conducted was in year 2000; thinning eliminated trees and families based on growth characters. The current number of trees is 620. Origin and number of families encompassed by the SSO are shown in Table 1. MLT was established in 2002. It is located near the F1 SSO. MLT encompasses a bulk of 64 open pollinated progenies of the F1 SSO. Parents of the progenies in the MLT were unknown.

B. DNA Extraction and Microsatellite Analysis

DNA was extracted from all trees in F1 SSO as parental population (N=620) and 32 best growth trees in the MLT as offspring population. The number of maternal trees for establishing CSO parental analysis was only based on 32 trees. The extraction was done following CTAB method (Shiraishi & Watanabe, 1995).

Multiplexed polymerase chain reactions (PCR) were developed in this study For the

Table 1. Number of families in the F1 SSO of *Acacia mangium*

Provenance	Population	Number of family	Family composition (%)
QLD	135K	36	6
	Cassowary	45	7
	ClaudieR	114	18
	ClaudieRI	38	6
	ClaudieRE	37	6
	Pascoe	28	5
	Poscoe	18	3
PNG	Arufi	30	5
	Bimadebun	28	5
	Biote	37	6
	Derideri	48	8
	Dimisisi	18	3
	Gubam	52	8
	Kini	46	7
	Wipim	45	7
Total		620	100

amplification, 5 ng DNA was used in the final volume of 10μL AmpliTaq Gold @360 master mix (Applied Biosystem; #ID:4398876). This study used 10 microsatellite loci i.e. Am014, Am136, Am326, Am341, Am387, Am429, Am435, Am460, Am465, and Am503 that developed for *A. mangium* (P. A. Butcher, Decroocq, Gray, & Moran, 2000). PCR involved a de-naturating step of 94oC for 5 min followed by 10 cycles PCR touchdown (94°C for 1 min, touchdown for change 1 min from 65°C till 55oC and 72°C for 1 min), followed by 25 cycles PCR (94°C for 1 min, 55°C for 1.5min and 72°C for 1.5min) and finally one cycle of 72°C for 7 min (Yuskianti & Isoda, 2012).

PCR product was mixed with HiDi Formamide (Applied Biosystem) and ROX 400HD size standard (Applied Biosystem) and denaturated at 95°C for 5 min before the products were analyzed using Gene Analyzer 3100 AVANT (Applied Biosystem). Fragment DNA patterns were analyzed by GeneMapper ver. 2.0 (Applied Biosystem).

C. Parent Identification

As described above this study was based on genotype data of 620 trees in F1 SSO as parental and 32 trees in MLT as offspring. Panmictic condition that estimated mating system status was conducted by comparing genetic diversity parameters between parental and offspring populations i.e. number of detected allele (N_A), expected heterozygosity (H_E), allelic richness (A_R) and coefficient inbreeding (F_{IS}). The AR was calculated with minimum number of samples i.e. 29 samples (58 gene copies) for each locus. The parameters were performed using FSTAT software (Goudet, 2001).

Direct parental analysis was estimated by comparing microsatellite data between 620 trees in F1 SSO and 32 best trees in MLT and calculated using Cervus 3.03 software (Kalinowski, Taper, & Marshall, 2007). Pollen dispersal distances were calculated manually by Pythagoras principles.

III. RESULTS AND DISCUSSION

A. Genetic Diversity within Parental and Offspring Population

Genetic diversity parameters for 10 microsatellite loci in parents and offspring are shown in Table 2. The number of detected alleles (N_A) was 160 in parental and 84 in offspring, respectively. For parental population, value of N_A per locus ranged between 8 and 25, and for offspring the value ranged between 3 and 13. The H_E ranged between 0.591 and 0.872

Table 2. Diversity parameters for six microsatellite loci in parental and offspring populations

Locus name	N		N_A		H_E		$A_{R[58]}$		F_{IS}	
	P	O	P	O	P	O	P	O	P	O
Am014	620	32	22	12	0.872	0.849	15.78	11.89	0.333*	0.007ns
Am136	620	32	20	7	0.820	0.767	10.19	6.98	0.066*	-0.019ns
Am326	620	32	19	13	0.816	0.806	11.52	12.60	0.301*	-0.124ns
Am341	620	32	8	3	0.591	0.415	4.39	3.00	0.227*	-0.012ns
Am387	620	32	15	10	0.814	0.802	8.75	9.74	0.166*	0.115ns
Am429	620	32	15	8	0.804	0.806	8.61	7.81	0.170*	-0.047ns
Am435	620	32	19	7	0.759	0.673	8.96	6.89	0.011ns	0.071ns
Am460	620	32	8	6	0.768	0.805	6.46	6.00	0.271*	0.319*
Am465	620	32	25	10	0.799	0.794	13.91	10.00	0.117*	0.348*
Am503	620	32	9	8	0.726	0.675	6.33	7.87	0.275*	-0.052ns
Mean	620	32	16	8.4	0.777	0.739	9.49	8.28	0.194*	0.066ns

Remarks: P: parental population, O: offspring population, N: number of samples, N_A: number of detected alleles, H_E: expected heterozygosity, A_R[58]: allelic richness, F_{IS}: coefficient inbreeding, level of significance: * $p<0.05$, NS: $p>0.05$

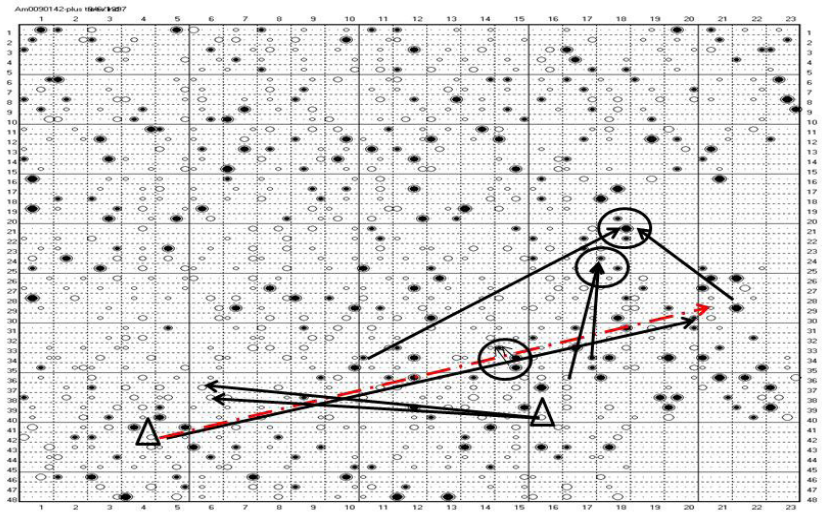

Figure1. Distribution of parent pairs within the SSO single population F1 of *A. mangium*. Arrow with line indicated pollen parents to the maternal trees

Remarks:

: Maximum pollen dispersed; Circles: a female tree pollinated by 2 male trees; Triangle: a male tree pollinated 2 female trees

for parental population, and 0.415 and 0.849 for offspring. The value of A_R ranged between 4.39 and 15.78 for parental population, and 3.00 and 12.60 for offspring. Deviations from Hardy-Weinberg expectations that measured the significant deficiency (F_{IS}) showed statistically high heterozygosity deficit for parental, but the value was insignificant for offspring.

The differences in genetic diversity parameters between parental population and offspring could be used as a mating system indicator within a seed orchard when polymorphism alleles were low (Chaix et al., 2003; Nurtjahjaningsih, Saito, Tsuda & Ide, 2007). Genetic diversity of offspring was higher than parental population under random mating/ panmictic equilibrium condition in seed orchard of *Pinus merkusii* (Nurtjahjaningsih et al., 2007) and *Eucalyptus grandis* (Chaix et al., 2003). The results in this study showed that parameters of genetic diversity in parental and best performer of offsprings were similar. Insignificant value of coefficient inbreeding in offspring population showed that mating system within the SSO was under panmictic equilibrium.

Synchronization flowering phenology is one of the important factors in random mating in order to produce quality and quantity seeds (El-Kassaby, Fashler, & Sziklai, 1984). Genetic variation increase when synchronization of flowering was achieved in Pseudotsuga menziesii seed orchard (El-Kassaby et al., 1998). However, this ideal condition rarely occurred due to differences in provenances/ geographical origins encompassing a seed orchard (Chaix et al., 2003; Moriguchi et al., 2004). Besides, flowering phenology also varied among individual trees or families within provenances (Burczyk & Chalupta, 1997). Unequal flowering phenology cause unbalances in gamete contribution and increase pollen contamination (Chaix et al., 2003; Moriguchi et al., 2004).

B. Parent Identification

Paternal analysis was conducted by comparing directly the genotypes of all trees in F1 SSO and the best 32 trees in the MLT. However to avoid reader misunderstanding, not all parent pairs were shown in the result. Figure 2 showed that, within the SSO, pollination pattern tended to have North direction. The behavior of the pollen donor to each maternal tree seemed to be independent of their position in the SSO.

Nevertheless, distances between the maternal trees and the pollen donor did not seem to have patterns; pollen donor trees could be from neighbor or distant trees. Based on position of paternal trees and maternal trees in the SSO, the distance of pollination ranged between approximately 15 to 150 m. Single pollen donor to the maternal trees seems to be the dominant pollination. However, interestingly, a maternal tree was pollinated by two pollen donor trees. In contrast, a pollen donor had pollinated two or more maternal trees.

Using 10 microsatellite markers, parent trees of plus trees in the MLT of *A. mangium* had been identified. The microsatellite markers has a powerful tool to identify the parent pair in the SSO that was characterized by value of non-exclusion probabilities (NEP:11x10-8). Specific microsatellite loci influence the value of exclusion probabilities (EP) that affect the accuracy of parental analysis (Chaix et al., 2003). Each microsatellite locus has many rare alleles, thus using this marker could be expected to have high value of EP (Dow and Ashley, 1998). A few number of microsatellite with high value of EP (>0.997) considered enough for paternity analysis (Chaix et al., 2003; Moriguchi et al., 2004).

Pollen dispersed randomly within the SSO (Figure 2). Pollen donor trees for each maternal tree seemed to be independent of their position in the SSO. Figure 2 also showed that pollen could be dispersed approximately 150 meters distances (red arrow). Previous study reported that pollen of *A. mangium* could be dispersed by only 40 m (Isoda, Yuskianti, & Rimbawanto, 2002). This distance was smaller than pollen contamination rate in *Eucalyptus grandis* seed orchard due to it was pollinated by another *E. grandis* planting test that was located 400 m away and one kilometer of *E. robusta*, respectively (Chaix et al., 2003). Moreover, pollen in coniferous species such as Pinus sylvestris could be dispersed within 30 km (Robledo-Arnuncio & Gill, 2005). Capability of dispersed pollen contributes to the design of the SSO. Pollen flow to the maternal tree might be affected by several factors. Individual trees which originate from same provenance expected to have similar pattern in flowering phenology (Burczyk & Chalupta, 1997). Distance pollen in pollination successes might be due to they have similar flowering characteristics. Moreover, insect pollinator works more effectively and is attractive in peak flowering and shiny season (Kang, 2000). Under flowering synchronization, reproductive success also could be restricted by prezygotic barriers (Larson et al., 2012) and assortative mating (mate choice) factors (Maroja et al., 2009). These barriers might be controlled by some genes within region and strongly prevent the reproductive success (Larson, White, Ross & Harrison, 2014). These gene barriers might determine the concept of combining ability i.e. general combining ability and specific combining ability of plant species.

IV. CONCLUSION

The study revealed that mating system within the SSO was near panmixia equilibrium. Paternal analyses showed a maternal tree of *A. mangium* tended to be pollinated by more than one paternal tree. Such maternal trees might be used to better design the clonal seed orchard in the future tree improvement strategy of this species. The mating system also showed that two good performance offsprings were fathered by one paternal tree; it showed that effect of inbreeding depression in *A. mangium* could be ignored. Flowering synchronization among the identified parent trees should be assessed in a future study, to maximize seeds production from the orchard.

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