DETECTION OF POLLEN FLOW IN THE SEEDLING SEED ORCHARD OF *Acacia mangium* USING DNA MARKER

Vivi Yuskianti[1] and Keiya Isoda[2,3]

ABSTRACT

Pollen pattern dispersal in seedling seed orchard (SSO) is an essential part of a tree-improvement program. Two SSOs of *Acacia mangium* in South Kalimantan and South Sumatra that represent similar resources in different environments were used in this study. Genotypes of all trees and seeds from a subset of 10 mother trees in each orchard were determined for 12 microsatellite loci, and parentage analysis was carried out. The results shows that the pollen dispersal pattern in both SSOs decrease with distance from mother tree. Patterns of pollen dispersal, dispersal distance and cumulative frequency of pollen dispersal distance were similar in both SSOs. Random pollen dispersal were found in both SSOs. About 80% of all crosses were found within a 40-m distance range with the most frequent pollination distance between mother tree and male male parents was 0-10 m. No self-pollinated seed was detected. Application of all these aspects found in this study such as random pollen dispersal and the effective pollen dispersal distance can be useful for establishing seedling seed orchard, clonal seed orchard and in other tree improvement activities of *A. mangium*.

Keywords: *Acacia mangium*, seedling seed orchard, pollen dispersal, effective distance, selfing ratio

I. INTRODUCTION

Understanding the mating system and patterns of pollen dispersal in tree species is important for conservation and natural resource management as well as for successful breeding program and domestication (Millar *et al.*, 2008). Mating system has been studying in *Acacia* species. Most of the studies found high levels of outcrossing in *acacia* species (Mandal and Ennos, 1995; Casiva *et al.*, 2004; George *et al.*, 2008; Millar *et al.*, 2008) with a little differences of a mixed-mating system in *A. sciophanes* and *A. anfractuosa* (Coates *et al.*, 2006). Study on mating system of *A. mangium* in natural populations showed that outcrossing rates in natural populations was ranged from complete selfing in outlying populations with low genetic diversity to complete outcrossing in the more variable New Guinea populations (Butcher *et al.*, 2004).

Genetic studies of mating system have been conducted using isoenzyme (Mandal and Ennos, 1995; Casiva *et al.*, 2004; Coantes *et al.*, 2006 and George *et al.*, 2006). Although population subdivision at isoenzyme loci can be used to estimate gene flow, it is difficult to determine the relative contributions of pollen movement and seed dispersal to overall migration rates (Dow and Ashley, 1996). Microsatellite markers are suitable for mating system studies as they are highly polymorphic, co-dominant, and considered to be selectively neutral (Tabbener and Cottrell, 2003; Millar *et al.*, 2008). PCR-based microsatellite analysis is also quick, and requires only small amounts of DNA (Hancock, 1999; Glaubitz and Moran, 2000). Therefore, microsatellite markers have been widely used for investigating mating system, patterns of pollen dispersal, pollen donor composition and parentage of saplings in many species (Dow and Ashley, 1996; Tabbener and

[1]Center for Forest Biotechnology and Tree Improvement, Jl. Palagan Tentara Pelajar Km 15, Purwobinangun, 55582 Sleman, Yogyakarta, Indonesia

[2]JICA Forest Tree Improvement Project Phase II, Jl. Palagan Tentara Pelajar Km 15, Purwobinangun, 55582 Sleman, Yogyakarta, Indonesia

[3]Curent 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:vivi_yuskianti@yahoo.com

Cottrell, 2003; Robledo-Arnuncio and Gil, 2005; Millar *et al.*, 2008; Hasegawa *et al.*, 2009).

A comprehensive tree improvement program for tropical fast growing species in Indonesia was launched in early 1992's (Nirsatmanto, 2005). This program was intended to cover up some commercial species: *A. mangium*, *A. crassicarpa*, *Eucalyptus pellita*, *E.urophylla*, and *Paraserianthes falcataria* (Hashimoto *et al.*, 1996). Among them, at present, *A. mangium* was the earliest progressed program with the improvement mainly subjected to meet the demand of supplying material for pulp and paper industry (Nirsatmanto, 2005). Through these programs, several seedling seed orchards (SSOs) of *A. mangium* have been established in several areas in Indonesia. The purpose of this study was to investigate the pattern of pollen dispersal in two seedling seed orchards (SSOs) i.e. AM006 group C (South Kalimantan) and AM004 group D (South Sumatra) in Indonesia. The results of this study can be used for evaluating and managing the existing seed orchards, designing the next SSOs, and also designing more efficient clonal seed orchards (CSOs) for improved seed production.

II. MATERIAL AND METHOD

A. Study Site and Sample Collection

Two first-generation (F1) seedling seed orchards (SSOs) in Indonesia and their offspring were used. One SSO is in South Kalimantan (AM006 Group C) and the other is in South Sumatra (AM004 Group D). AM006 Group C is located in Sebuhung village, sub-district of Lorong, district of Tanah Laut, South Kalimantan province, Kalimantan Island; AM004 Group D is located in Talang Ubi sub-district, district of Muara Enim, South Sumatra province, Sumatra Island (Table 1). These SSOs were selected because they represent similar resources in different environments and should provide an indication of consistency in the mating system in *A. mangium*.

More than 60 families from several Australian

Table 1. Detailed information about the two seedling seed orchards (SSO) used for the mating system study in *A. mangium*

Information	South Kalimantan (AM006) SSO[a]	South Sumatra (AM004) SSO[b]
Species	*A. mangium* (C)	*A. mangium* (D)
Country	Australia	Australia
Provenance	Claudie River, etc	Claudie River, etc
Seed Sowing data	September 4, 1993	September 29, 1993
Planting Date	December 23-24, 1993	January 7-9, 1994
Spacing	4 x 2 m	4 x 2 m
Ground cultivation	Plowing 2 ×	Plowing 1 ×
Fertilizer	NPK 2 ×/year	TSP 1 × and urea 1 ×
Vegetation cover	*Imperata cyclindrica*	*Imperata cylindrica*
Experimental site		
Latitude	3'58' to 4'08' South	4' South
Longitude	114'37' to 114'42' East	104' East
Elevation	15-150 m	80 m
Climate type	B (Schmidt and Ferguson)	A (Schmidt and Ferguson)
Annual rainfall	2730 mm	2781.23 mm
Max. temperature	33 ^{0}C	33 ^{0}C
Min. temperature	23 ^{0}C	24 ^{0}C
Wind speed	Middle	Low
Slope	0-15%	5%
Soil type	Ferralsols	Acrisols
Site details		
Compartment	Block HoD/Sebuhur	Block Se{}untung II/Petak 37
Site Area	2.00 ha	0.96 ha
Design	RCBD	RCBD
Number of trees/plot	4	4
Number of families	63	63
Number of replication	10	8

Note: [a]Kurinobu *et al.,* 1994a; [b]Kurinobu *et al.,* 1994b

provenances has been used for development of F1 generation. Progeny test was conducted in both SSOs: AM006 Group C and AM004 Group D. Four trees per plot of each family were planted initially and within-plot selection was applied, after which only one tree per plot remained. All trees in each SSO were used to determine male parent genotypes; there were 379 trees in Group C AM006 South Kalimantan and 251 trees in AM004 South Sumatra.

All the material used in this study were collected in 2002. Two or three leaves from each tree from these orchards were collected and put in an envelope with sufficient proportion of silica gel inside. The silica gel was replaced many times to dry the leaves and minimize decay or associated fungi. For offspring, seeds were collected from ten mother trees (Butcher *et al.*, 2004; Millar *et al.*, 2008) at each site. The mother trees were selected based on their position in the orchard. These positions were expected to represent anticipated trends in the mating system in each orchard. From each mother tree, representative pods from throughout the crown were collected; twenty seeds were randomly selected from these bulky pods.

B. DNA Extraction

Genomic DNA from both leaves and seeds was isolated using a modified CTAB method (Shiraishi and Watanabe, 1995). The extraction procedure for each differed. For leaves, 100 mg were cut into small pieces and ground to a powder under liquid nitrogen in a pestle and mortar. The powder was then put into a 2.0 ml microtube and one ml of extraction buffer (100 mM Tris-HCl pH 9.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 0.5% ß-mercaptoethanol) added. For seeds, every fresh seed was put into a tea bag, boiled in water for 10 min, and the seed coat removed manually. The naked seed was placed in a 1.5 ml microtube, incubated for 30 min at 65ºC in 0.6 ml extraction buffer (100 mM Tris-HCl pH 9.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 0.5% ß-mercaptoethanol) and then ground using a pellet pestle. The ground

material of both leaves and seeds was then incubated in their respective microtube at 65ºC for 1 h. Chloroform was used twice to wash the supernatant containing DNA. Sodium acetate and isopropanol were used to precipitate the DNA. A DNA pellet was made by centrifuging the tube at 15,000 rpm. The pellet was washed with 70% ethanol and then distilled water added to redissolve the DNA.

C. PCR Analysis

Twelve microsatellite markers (Butcher *et al.*, 2000) were used for genotype analysis. For each marker, a fluorescent-labelled primer and a non-labelled reverse primer were synthesized for PCR analysis. PCR analysis was carried out in a 10 μl reaction containing 1 PCR buffer (Perkin-Elmer, Applied Biosystems, USA), 1.5 to 3.0 mM $MgCl_2$ and 0 or 1 % formamide (Table 2), 200 μM each dNTP, 0.5 μM each primer, 0.5 unit *AmpliTaq Gold* DNA polymerase (Perkin-Elmer, Applied Biosystems, USA), and 25 ng template DNA. The optimized $MgCl_2$ concentration and the addition of formamide for each marker (Table 2) were used to obtain the minimum or maximum single nucleotide addition caused by the *Taq* DNA polymerase.

Amplification was performed in a GeneAmp PCR System 9700 (Perkin-Elmer, Applied Biosystems, USA) at 94˚C for 10 min, 35 cycles at 94˚C for 30 s, 50-60˚C for 30 s, 72˚C for 60 s, followed by 1 min at 72˚C for the extension step. Touch-down PCR (Don *et al.*, 1991) was used for PCR analysis. The annealing temperature for the initial cycle was 65˚C, which was then decreased by 1˚C/cycle for the following nine cycles. After the 10th cycle, the annealing temperature was fixed at 55˚C. PCR products were electrophoresed using an ABI 310 Genetic Analyzer (Perkin-Elmer, Applied Biosystems, USA). The lengths of PCR products were determined using GeneScan and Genotyper software (Perkin-Elmer, Applied Biosystems, USA) and interpreted to the alleles.

Table 2. Details of 12 microsatellite markers used in our study

No	Marker	Primer sequences (5'- 3')	Repeat motif	MgCl$_2$ concentration
1	Am 014	GTA CTA ACG TTG CTA TAT GAG AAA GG CTG GTT GTT CGC TTA TAT GG	$(ATAC)_3(AC)_{26}(AT)_3$ $(GTAT)_2(AT)_3AC$	2.5 mM
2	Am 041	TAG GCT AAT GGT CAT ATT CCT AG AGA GAT AGG GGT ACA CAC TAA AAA AAC	$(GT)_{36}$	3.0 mM + 1 % formamide
3	Am 136	CCC ATT GCC GTT TCT TTG GCA TTT CCC TTG GAA CAG TC	$(CT)_{20}$	3.0 mM + 1 % formamide
4	Am 326	GGA CCA AAC TTA TGC AAC ACC GCA TCA ATG TAC TAA ACC ATT TCC	$(CA)_{20}$	2.5 mM
5	Am 341	CCA TTC GAG CAT CCT AAG AG CGT ATG GCT GAG CTA CTT AAT CA	$(CA)_{12}(TA)_2$	1.5 mM
6	Am 387	TGA TAC AAG GGA AGA CAG AGT GG CCA ACT CAA AAC CTG ACA ACG	$(AT)_2(GT)_2(AT)_2(GT)_{17}-(TA)_8$	1.5 mM
7	Am 429	CCT TCT TCT CTC ATC TAC CAA ACC CCC ACA TCA TCA CTC ACA ACT	$(GT)_{16}$	1.5 mM
8	Am 435	ACC CTT TAT TTC TCA CAC GGA ACA GAA GAA GAT GCA AAG AAG G	$(CT)_5,CC(CT)_2(CA)_9(CACT)_3$	2.5 mM
9	Am 436	ATG GAT CTT GTC CTT ATC TTG A GGG CCA ATT TGA GTT TGG AA	$(TG)_{14},T$	2.5 mM
10	Am 460	CAC TAA TTG CTC ACA CAT TCC A ATT CAT AGC CTC TCC CTT CAG	$(AT)_4,(GT)_{12},(GGAAT)_2,(GT)_2$	1.5 mM
11	Am 465	TGG GTA TCA CTT CCA CCA TT AGG CTG CTT CTT TGT GCA GG	$(AC)_{23}$	3.0 mM + 1 % formamide
12	Am 503	GTA TGA GTT CCA GTC CTA CCA TCA CAG TCC GGT TTT TGC TGT CA	$(TG)_{13},(TG)_8$	1.5 mM

D. Data Analysis

The level of genetic diversity and parentage analysis of the two *A. mangium* SSOs of was calculated using Cervus 3.0 that was developed by Marshall *et al*. (1998) and revised by Kalinowski *et al*. (2007). The expected heterozygosity in the software was calculated using an unbiased formula from allele frequencies assuming Hardy-Weinberg equilibrium (Nei, 1987), whereas PIC that measure of informativeness related to expected heterozygosity and likewise was calculated from allele frequencies (Botstein *et al*., 1980; Hearne *et al*., 1992). The candidate male parent as pollen donor of each offspring was determined after following some steps of parentage analyis on Cervus 3.0 software. The dispersal of pollen donor from mother tree was then drawn in a map of each orchard and their distance was manually calculated. The process of drawing the position of male parent in the map and the prediction of their distance was simplify by the use of a map that was developed using a program for making an experimental design of family plots on SSOs, Design.exe (Kawasaki *et al*., 2000).

III. RESULT AND DISCUSSION

A. Genetic Diversity

The twelve microsatellite markers in *A mangium* was highly variable. AM004 Group D from South Sumatra has higher number of alleles than AM006 Group C from South Kalimantan. The number of alleles (*A*) varied from 4-21 alleles per locus with an average 8.75 alleles in AM004 Group D and 7.67 alleles in average with *A*=3-17 alleles per locus in AM006 Group C from South

Kalimantan) (Table 3). The observed heterozygosity (H_o) in both orchards was lower than its expected heterozygosity (H_e). The level of genetic diversity in both orchards was higher than found in planted stand of *Acacia saligna* subsp. *saligna* (Millar *et al.*, 2008). The both orchards established using the similar provenances (Australia) so they have relatively similar level of genetic diversity (H_e=0.610 in AM004 Group D and H_e=0.606 in AM006 Group C).

In general, high polymorphic information content (PIC) (0.554 in AM006 Group C and 0.0552 in AM004 Group D in average) was found in both orchards. The PIC varied from 0.267 to 0.710 and 0.357 to 0.783 in AM006 Group C South Kalimantan and AM004 Group D South Sumatra, respectively. The lowest and the highest

PIC was occured in primer Am341and Am041. (Table 3). Frequency of null alleles ($\pm$ 0.051 in average) was low in both orchards (Table 3). The negative value of null allele that mean an excess of observed heterozygote genotypes was found for primer Am435 in AM006 Group C South Kalimantan. Further analysis revealed 14,87% and 15.16% of an excess of homozygotes for primer Am460 in AM006 Group C and AM004 Group D, respectively. A locus with a large positive estimate of null allele frequency does not necessarily imply that a null allele is present, however, it usually shows a characteristic pattern of repeated homozygote-homozygote mismatches in known parent-offspring relationship (http://www.fieldgenetics. com).

Table 3. Utility of 12 microsatellite loci for two SSOs of *A. mangium*

Locus	A	H_o	H_e	PIC	Null
AM006 Group C South Kalimantan					
Am014	11	0.590	0.694	0.665	0.0830
Am041	17	0.662	0.732	0.710	0.0473
Am136	7	0.660	0.726	0.677	0.0500
Am326	11	0.511	0.543	0.519	0.0291
Am341	3	0.300	0.316	0.267	0.0251
Am387	7	0.552	0.648	0.578	0.0798
Am429	9	0.643	0.671	0.615	0.0207
Am435	6	0.682	0.654	0.599	-0.0172
Am436	6	0.458	0.501	0.427	0.0433
Am460	6	0.544	0.731	0.694	0.1487
Am465	4	0.475	0.517	0.406	0.0420
Am503	5	0.470	0.538	0.489	0.0666
All loci	7.67	0.546	0.606	0.554	0.0515
AM004 Group D South Sumatra					
Am014	21	0.597	0.672	0.643	0.0518
Am041	15	0.684	0.803	0.783	0.0777
Am136	7	0.613	0.671	0.628	0.0391
Am326	10	0.457	0.495	0.461	0.0373
Am341	4	0.405	0.461	0.357	0.0632
Am387	11	0.583	0.626	0.567	0.0315
Am429	10	0.621	0.670	0.617	0.0379
Am435	6	0.594	0.606	0.563	0.0119
Am436	4	0.426	0.490	0.413	0.0719
Am460	6	0.524	0.711	0.666	0.1516
Am465	6	0.509	0.535	0.425	0.0217
Am503	5	0.577	0.585	0.499	0.0061
All loci	8.75	0.549	0.610	0.552	0.0501

Results are presented for parental and progeny cohorts combined. A=Number of alleles, H_o=observed heterozygosity, H_e=expected heterozygosity, PIC=polymorphic information content, Null=estimated frequency of null alleles. A negative number denotes an excess of observed heterozygotes

B. Pollen Dispersal

Twelve microsatellite markers were sufficient to establish the genotype of all trees in each SSO; however the success ratio for establishing seed genotype was variable. For the 20 seeds from each mother tree, 18-19 seeds in a total of 188 seeds in AM006 group C (South Kalimantan) and 19-20 seeds in a total of 192 seeds in AM004 group D (South Sumatra) were identified to genotype (data not shown); 112 seeds in AM006 and 142 seeds in AM004 were matched with their mother-tree genotype. Mismatching between mother tree and seeds was detected in 77 seeds in AM006, and 50 seeds in AM004. Detection power in AM006 was 32 seeds with a frequency of 28.8 % and in AM004 was 60 seeds with a frequency of 42.3 %.

Pollen dispersal is a major component of gene flow in plant populations (Austerlitz *et al.*, 2004; Robledo-Arnuncio and Gil, 2005). Pollen donors can dispersed randomly around mother tree and crossing could be occured from all direction (Figure 1), the similar trend that was found in bur oak, *Quercus macrocarpa* (Dow and Ashley, 1996) and a planted stand of *A. saligna* subsp. *saligna* (Millar *et al.*, 2008).
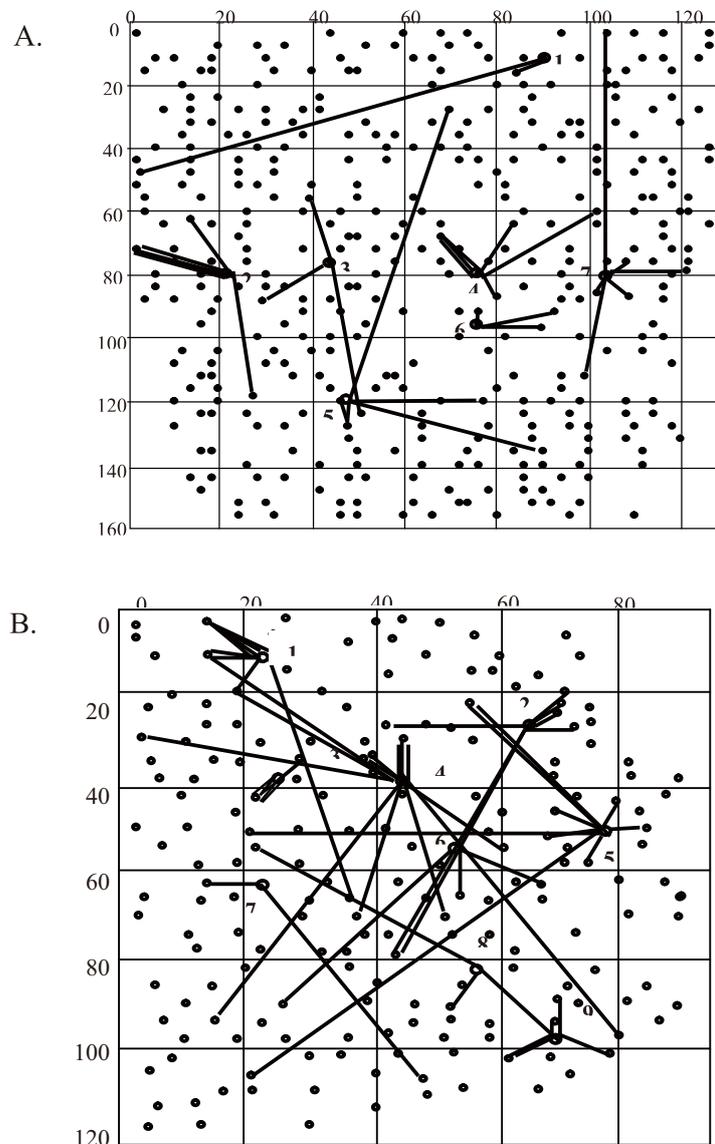

Figure 1. Pollen dispersal in AM004 Group D (South Sumatra) SSO (A) and AM006 Group C (South Kalimantan) SSO (B). The selected trees are indicated with small circles. The mother trees were indicated with large circles. Pollen dispersal is indicated with lines

Pattern of pollen dispersal in both SSOs of *A. mangium* was random, however, we also found multiple paternity/correlated outcrossing wherein some of the detected paternal trees were to be the pollen donor of several seeds (Table 4). It was 3 trees (9.4%) that were pollen donors of 8 seeds (25%) in South Kalimantan, whereas in South Sumatra, there were nine trees (15 % of all detected paternal trees) as pollen donor of 27 seeds (45% of all pollen donors). Contribution of pollen from neighboring trees was high; 7 trees (21.8%) in AM006 and 16 trees (26.7%) in AM004 (Table 4). They were pollen donors of nine seeds (28.1% from all pollen donors) in

South Kalimantan and 29 seeds with frequency 48.3 % from all pollen donors in South Sumatra. The correlated mating (George *et al.*, 2008; Coantes *et al.*, 2006; Millar *et al.*, 2008) was more likely to be due to nature of *Acacia* floral biology (George *et al.*, 2008; Millar *et al.*, 2008). Pollen in *Acacia* is presented in the form of polyads (fused pollen grains), so all insects that forage for polyads will have immediate access to respective stigmas (Coantes *et al.*, 2006). In *A. saligna*, the flowers are grouped into complex inflorescences, and pollen from the same paternal tree may regularly fertilize more than one flower within a cluster (Millar *et al.*, 2008). Pollinator will concentrate on more

Table 4. Pollen donors in AM006 group C (South Kalimantan) SSO (A) and AM004 group D (South Sumatera) SSO (B)

No	Mother Tree ID	N pollen donor	Multi contributions [1]		N Neighbor [2]		Self
			Tree ID	N. seed	Tree ID	N.seed	
A.	AM006 group C (South Kalimantan) SSO						
1.	AM006 03/45	6	AM006 03/12	3	AM006 03/68	1	0
2.	AM006 04/47	5	AM006 04/67	2	AM006 04/67	2	0
3	AM006 03/47	8	-	-	AM006 03/36	2	0
					AM006 03/71	1	
					AM006 03/59	1	
4	AM006 03/29	3	-	-	-		0
5	AM006 04/02	3	-	-	AM006 03/78	1	0
6	AM006 03/41	5	AM006 03/14	3	-		0
7	AM006 01/28	2	-	-	AM006 01/41	1	0
	Total	32	3 (9.4%)	8 (25%)	7 (21.8%)	9 (28.1%)	0
B.	AM004 group D (South Sumatera) SSO						
1.	AM004 06/19	8	AM004 06/18	2	AM004 06/18	2	0
			AM004 06/03	4			
2.	AM004 06/54	7	AM004 06/46	2	AM004 06/46	2	0
			AM004 08/17	2			
3	AM004 06/80	8	AM004 07/08	7	AM004 07/08	7	0
					AM004 06/71	1	
4	AM004 07/11	14	AM004 07/02	4	AM004 07/02	4	0
			AM004 07/01	2	AM004 07/01	2	
5	AM004 07/33	9	AM004 06/44	2	AM004 07/21	1	0
					AM004 07/22	1	
					AM004 07/53	1	
					AM004 07/34	1	
					AM004 07/32	1	
6	AM004 07/41	3	-	-	AM004 07/50	1	0
7	AM004 07/57	2	-	-	AM004 07/56	1	0
8	AM004 08/25	3	-	-	AM004 08/42	1	0
9	AM004 08/60	6	AM004 08/66	2	AM004 08/52	1	0
					AM004 08/66	2	
	Total	60	9(15%)	27 (45%)	16(26.7%)	29 (48.3%)	0

Note:
[1] Multi contributions of trees that were male parents;
[2] The number (N) of male parents adjacent to the mother tree producing seeds

rewarding patches, and when pollinators visit more rewarding patches, they will travel less far to the next flower or plant (Richards, 1997). Flowering time will also influence the trend. Butcher (2002) found no evidence of selfing in location where the majority of trees flowered synchronously and seed orchard established from the same source population. Selfing should be avoided because it usually performs negative effect and inbreeding depression. Study on effect of inbreeding on *A. mangium* found that self-fertilized individuals were significantly (P<0.001) slower-growing than were outcrossed individuals (Harwood *et al.*, 2004).

The most frequent pollination distance between mother tree and male parent was 0-10 m in both SSOs; distances of 10-20 m, 20-30 m and 30-40 m were associated with a lower frequency of pollination. In the AM006, the number of crosses was 28.1 %, 25 %, 15 % and 9.4 % at 0-10 m, 10-20 m, 20-30 m and 30-40 m distance, respectively. In AM004, the trend was similar but most of the crossing was in 0-10 m range; the other ranges showed very low frequency (Figure 2). The pollinator movement might have contributed to these patterns. The bee genus *Trigona* are the most numerous insect visitors to *A. mangium* flowers and had the highest numbers of polyads on their hairy bodies (Sedgley *et al.*, 1991). For bee-pollinated plants, it is usual to find at least 80% of flights are less than 1 m in distance, and 99% are less than 5 m, though the nature of pollinator behaviour that occasional grains may travel much further (Richards, 1997).

Furthermore, travel will be much shorter in areas of high plant density such as in AM004 group D (established in 0.96 ha area with 251 trees) than in AM006 Group C that has lower density (190 trees ha^{-1}). As explained in Richards (1997), in sparsely populated areas, pollen travel will, by definition, be more distant, however, the number of plants which the pollen may reach may be no greater than in dense populations, and indeed may well be less. Sparse area of dense population will also receive far fewer pollinator visits, as these areas will form patches of low reward, and thi will minimize gene travel in these areas (Richards, 1997).

Study in *Acacia* sp. showed that most of *Acacias* are outcrossing species. It was found in *A. nilotica* ssp. *kraussiana* (Mandal and Ennos, 1995), *A. saligna* subsp. *saligna* (Millar *et al.*, 2008), mixed mating with preferential outcrossing in *A. saligna* (George *et al.*, 2006) and high levels of outcrossing with little variation in the outcrossing rate between populations in *A. auriculiformis* and *A. crassicarpa* (Moran *et al.*, 1989b). Our study suggest a possible of mixed mating with predominantly outcrossing in *A. mangium*. It was supported by our data that found no self-pollinated seed (Table 4), the presence of multiple paternity/correlated outcrossing (Table 4) and high crossing in close area (Figure 2) with neighbour trees (Table 4). Previous *A. mangium* study showed that this species has flexible mating system with its outcrossing rates in natural populations range from complete selfing in outlying populations with low genetic diversity to complete outcrossing in the
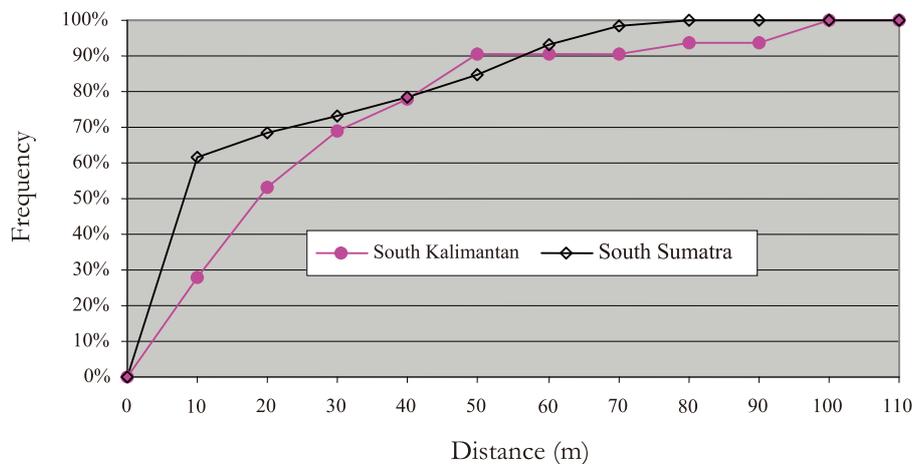

Figure 2. The cumulative frequency of pollen dispersal distance in the AM006 group C (South Kalimantan) and AM004 group D (South Sumatra) SSO

more variable New Guinea populations (Butcher *et al.*, 2004). The trend of mating with their nearest neighbours can result in significant inbreeding. Computer simulations study of nearest-neighbor pollination in outcrossing plant species found that this restricted gene flow caused inbreeding, a rapid increase in homozygosity, and striking microgeographic differentiation of the populations (Turner *et al.*, 1982).

The design of an efficient tree breeding strategy will be greatly influenced by our knowledge and through understanding of the mating systems (Mandal and Ennos, 1995). Our analysis in *A. mangium* showed that about 80 % of all crosses were found within 40-m distance range in both SSO (Figure 2). The similar result was also found in other studies in planted stand of *A. saligna*. The average pollen dispersal distance was 37 m with the majority of progeny sired by paternal trees within a 50-m neighborhood of the maternal tree (Millar *et al.*, 2008). The both study using *Acacia* species in planted area such as seedling seed orchard will gives an impact for designing seed orchard, clonal seed orchard etc. The result can be applied for such as application of target trees in 40-m distance range in order to increase chances of mating between the expected trees.

Seeds within a pod are full sib relationship (Isoda *et al.*, 2002) so the estimated haplotypes was searched from the genotype data of all the trees in the SSO. In most cases, only one tree will match to the estimated haplotype, however, there some haplotypes were not found in the SSO. This is happened because the pollen may come from trees outside the SSO. Studies showed that 62% in neo tropical tree (Latouche-Halle *et al.*, 2004) and 66.1% in norway spruce (Piotti *et al.,* 2009) of the pollen contributing to seed came from outside the study plot. Minimum isolation distance is needed to reduce pollen contamination from outside the SSO. Timyan (1999) predicted that a distance of up to 100 m should be sufficient to isolate the orchard from such sources of contamination. The orchards used in our study adopted a 100-200 m isolation distance to avoid pollen contamination (Nirsatmanto, pers. comm.). The isolation distance was expected can reduce pollen contamination, however, as our study still found male parent in the range of 100 m from mother

tree and high mismatched in known parent and offspring so the possibilities of minimum isolation distance to reduce pollen contamination from outside the SSO could not be predicted. More studies using different distance as an isolation area in *A. mangium* is expected can resolve effective isolation distances in *A. mangium* to reduce pollen contamination from outside SSO.

IV. CONCLUSION

The mating system study using 12 microsatellite markers found high outcrossing with low selfing evidence was found in both SSO. Pollen dispersed randomly among the trees in each SSO and mating mostly occurs in close distance. Application of all these aspects of mating systems found in this study such as random pollen dispersal and the effective pollen dispersal distance can be used for establishing seedling seed orchard, clonal seed orchard and in other tree improvement activities of *A. mangium*.

V. ACKNOWLEDGEMENTS

REFERENCES

Austerlitz, F., Dick, C. W., Dutech, C., Klein, E. K., Oddou-Muratorio, S., Smouse, P. E., et al. (2004). Using genetic markers to estimate the pollen dispersal curve. *Molecular Ecology, 13*, 937-954.

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. *American Journal of Human Genetics, 32*, 314-331.

Butcher, P. (2002). Molecular breeding of tropical trees. *Proceeding of the International Conference on Advances in Genetic Improvement of Tropical Tree Species, 1-3 October 2002 Yogyakarta, Indonesia.* Yogyakarta: Centre for Forest Biotechnology and Tree Improvement.

Butcher, P. A., Decroocq, S., Gray, Y., & Moran, G. F. (2000). Development, inheritance and cross-species amplification of microsatelite markers from *Acacia mangium. Theor Appl Genet, 101,* 1282-1290.

Butcher, P., Harwood, C., & Quang, T. H. (2004). Studies of mating systems in seed stands suggest possi bl e causes of variable outcrossing rates in natural populations of *Acacia mangium. Forest Genetics, 11*(3-4), 303-309.

Casiva, P. V., Vilardi, J. C., Cialdella, A. M., & Saidman, B. O. (2004). Mating system and population structure of *Acacia aroma* and *A. macracantha* (Fabaceae). *American Journal of Botany, 91*(1), 58-64.

Coantes, D. J., Tischler, G., & McComb, J. A. (2006). Genetic variation and the mating system i n the rare Acacia sciophanes compared with its common sister species *Acacia anfractuosa* (Mimosaceae). *Conservation Genetics, 7,* 931-944.

Don, R. H., Cox, P. T., Wainwright, B. J., Baker, K., & Mattick, J. S. (1991). 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research, 19,* 4008.

Dow, B. D., & Ashley, M. V. (1991). Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa. Molecular Ecology, 5,* 615-627.

George, N., Byrne, M., & Yan, G. (2008). Mixed mating with preferential outcrossing in *Acacia saligna* (Labill.) H. Wendl. (Leguminosae: Mimosoidae). *Silvae Genetica, 53*(3), 139-145.

Glaubitz, J. C., & Moran, G. F. (2000). Genetic tools: the use of biochemical and molecular markers. In A. Young, D. Boshier , & T. Boy l e (Eds.), *Forest Conservation Genetics, Principles and Practice.* Australia: CSIRO Publishing.

Hancock, J. M. (1999). Microsatellite and Other Simple Sequences: Genomic Context and Mutational Mechanisms. In D. B. Goldstein, & C. Schlotterres (Eds.), *Microsatellites, Evolution and Appllications.* New York: Oxford University Press.

Harwood, C. E., Thinh, H. H., Quang, T. H., Butcher, P. A., & Williams, E. R. (2004). The effect of inbreeding in early growth of *Acacia mangium* in Vietnam. *Silvae Genetica, 53*(2), 65-69.

Hasegawa, Y., Suyama, Y., & Sewa, K. (2009). Pollen donor composition during the early phases of reproduction revealed by DNA genotyping of pollen grains and seeds of *Castanea crenata. New Phytologist, 182,* 994-1002.

Hashimoto, K., Kurinobu, S., & Suhendi, H. (1996). Establishment of seed sources of tropical tree species in Indonesia. *Proceeding of the QFRI-IUFRO Conference, Caloundra, Australia,* (pp. 370-371).

Hearne, C. M., Ghosh, S., & Todd, J. A. (1992). Microsatellites for linkage analysis of genetic traits. *Trends in Ecology and Evolution, 8,* 288-294.

Isoda, K., Yuskianti, V., & Rimbawanto, A. (n.d.). Verification of full-sib relationship of seeds within a pod of *Acacia mangium* sing parentage analysis by microsatellite markers . *Proceeding of the International Conference on Advances in Genetic Improvement of Tropical Tree Species, 1-3 October 2002. Yogyakarta, Indonesia.* Yogyakarta: Centre for Forest Biotechnology and Tree Improvement.

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program cervus accomodates genotyping error increases success in paternity assignment. *Molecular Ecology, 16,* 1099-1106.

Kawasaki, H., Kurinobu, S., & Leksono, B. (2000). *Manual of the "Design.exe" for making an experimental design of family plots on seedling seed orchards. FTIP-P2-No.17.* Yogyakarta.

Kurinobu, S., Nirsatmanto, A., & Susanto, M. (1994a). *General information of seed source stablishment of* Acacia mangium *in South Kalimantan (Fiscal year 1993/1994). FTIP No. 22.* Yogyakarta.

Kurinobu, S., Nirsatmanto, A., & Susanto, M. (1994b). *General information of seed source stablishment of* Acacia mangium *in South Sumatra (Fiscal year 1993/1994). FTIP No.23 .* Yogyakarta.

Latouche-Halle, C., Ramboer, A., Bandou, E., Caron, H., & Kremer, A. (2004). Long distance pollen flow and tolerance to selfing in a neotropical tree species. *Molecular Ecology, 13,* 1055-1064.

Mandal, A. K., & Ennos, R. A. (1995). Mating system analysis in a natural population of *Acacia nilotica* subspecies *kraussiana. Forest Ecology and Management, 79,* 235-240.

Marshall, T. C., Slate, J., Kruuk, L. E. B., & Pemberton,

J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology, 7*, 639-655.

Millar, M. A., Byrne, M., Nuberg, I., & Sedgley, M. (2008). High outcrossing and random pollen dispersal in a planted stand of *Acacia saligna* subsp. saligna revealed by paternity analysis using microsatellites. *ree genetics & Genomes, 4*, 367-377.

Moran, G. F., Muona, O., & Bell, J. C. (1989). *Acacia mangium*: a tropical forest tree of the oastal lowlands with low genetic diversity. *Evolution, 43*(1), 231-235.

Moran, G., Muona, O., & Bell, J. C. (1989). Breeding systems and genetic diversity in *Acacia auriculiformis* and *A. crassicarpa. Biotropica, 21*(3), 250-256.

Nei, M. (1987). *Molecular evolutionary genetics*. New York: Columbia University Press.

Nirsatmanto, A. (2005). *Study on statistical genetic analysis, and the application to the reeding in* Acacia mangium (Doctoral Thesis). Japan: Kyushu University.

Piotti, A., Leonardi, S., Piovani, P., Scalfi , M., & Menozzi, P. (2009). Spruce colonization at treeline: where do those seeds come from? *Heredity, 103*, 136-145.

Richards, A. J. (1997). *Plant Breeding Systems* (2nd ed.). London: Chapman & Hall.

Robledo-Arnuncio, J. J., & Gil, L. (2005). Pattern of pollen dispersal in a small population of Pinus sylvestris L. revealed by total-exclusion paternity analysis. *Heredity, 94*, 13-22.

Sedgley, M., Khen, C. V., Smith , R. M., & Harbard, J. (1991). Insect visitors to flowering branches of *Acacia mangium* and *Acacia auriculiformis*. In L. T. Carron, & K. M. Aken (Ed.), *Breeding Technologies for Tropical Acacias. ACIAR Proceedings No. 37*. Canberra.

Shiraishi, S., & Watanabe, A. (1995). dentification of chloroplast genome between *Pinus densiflora* Sieb. et Zucc. and *P. thunbergii* Parl. based on the polymorphism in rbcL gene. *Journal of the Japanese Forestry Society, 77*(5), 429-436.

Tabbener , H. E., & Cottrell, J. E. (2003). The use of PCR based DNA markers to study the paternty of poplar seedlings. *Forest Ecology and Management, 179*, 363-379.

Timyan, J. C. (1999). *Management plan for improved tree seed production from orchards and progeny/provenance trials in Haiti*. Port-au-prince, Haiti: Pan American Development Foundation/PLUS Project 27.

Turner, M. E., Stephens, J. C., & Anderson, W. W. (1982.). Homozygosity and patch structure in plant populations as a result of nearest neighbor pollination. *Proc. Natl. Acad. Sci, 79*, 203-207.