COMPARATIVE STUDY OF RECIPROCAL CROSSING FOR ESTABLISHMENT OF *ACACIA* HYBRIDS

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KATO K, YAMAGUCHI S, CHIGIRA O & HANAOKA S. 2013. Comparative study of reciprocal crossing for establishment of *Acacia* hybrids. Using two *Acacia mangium* and five *A. auriculiformis* clones, *Acacia* hybrids were reciprocally artificially pollinated by tube pollination to determine which cross combination was more appropriate. Artificial pollination was also conducted on the intraspecific pollination of each clone. Pollen germination percentage and number of flowers for each clone were also investigated. The pollen germination percentage showed significant difference between the clones but no significant difference between species. The number of flowers per spike on *A. mangium* was almost double that on *A. auriculiformis*, but this difference did not affect the productivity of seeds. Although seed productivity was not different between reciprocal crossings, number of mature seeds, seed germination percentage and number of seedlings acquired were significantly higher when *A. mangium* was used as the female adult and also varied greatly among cross combinations. Seed productivity was also low on the intraspecific pollination of *A. auriculiformis*. By DNA analysis, 0.9% of seedlings produced by interspecific pollination were not hybrid. From these results, we suggested that three points for breeding programme of *Acacia* hybrids, namely, availability of each reciprocal crossing, caution for using *A. auriculiformis* clones in interspecific pollination and possibility of self-compatibility by tube pollination.

Keywords: *Acacia mangium*, *Acacia auriculiformis*, pollen germination, seed productivity, breeding programme

INTRODUCTION

Acacia hybrids formed by crossing *A. auriculiformis* and *A. mangium* were first discovered in the field in Sabah, Malaysia in the 1970s (Pedley 1978). Due to their morphological traits such as growth rate, stem straightness, wood density and resistance to pests and diseases, besides showing superior silvicultural characteristics over both parents (Kha 1996), these hybrids have been subjected to mass propagation by rooted cutting. Their wood properties have also been widely studied (Kim et al. 2009).

Artificial hybridisation using both tree species has been carried out (Sedgley et al. 1992a, Kato et al. 2012) and the establishment of hybrids has been successful. In a study of reciprocal crossing of artificial pollination between *A. auriculiformis* and *A. mangium*, it was reported that height of surviving hybrid seedlings after six months is not different between reciprocal crossings (Sedgley et al. 1992b). However, although the combination of female and male parents is not consistent between reciprocal crossings, a greater number of mature seeds is produced when *A. auriculiformis* is used as female parent (Sedgley et al. 1992a). The difference in the number of mature seeds between reciprocal crossings for interspecific pollination is also reported on other tree species (Hauser et al. 1998, Lopez et al. 2000, Tanihaya et al. 2014). On the other hand, no buds were formed when one specific *A. auriculiformis* tree was used as female parent although the percentage of flowers with penetrated ovules was 50% (Sedgley & Harbard 1993). The number of flowers per spike on *A. mangium* is almost double that on *A. auriculiformis* (Sedgley et al. 1992b), suggesting that a lot of seeds are potentially produced on an *A. mangium* spike. Thus, more precise data is required to evaluate each cross combination to detect which combination is effective or whether the produced seeds vary within cross combinations for acquiring more hybrid seeds.

In this study, the reciprocal crossing of interspecific and intraspecific pollination was artificially conducted using two *A. mangium* and five *A. auriculiformis* clones. A pollen germination test and the counting of the number of flowers on spikes for each clone were also conducted. Subsequently, the percentage of spike-forming pods, number of pods per spike, number of mature and immature seeds produced per spike and seed germination percentage per spike were assessed. To detect the percentage of self-compatibility, DNA of seedlings harvested was analysed. Finally, the number of hybrid seedlings obtained per spike was compared between reciprocal crossings. Results of this study will provide valuable information for conducting artificial pollination to establish *Acacia* hybrid for breeding programme.

MATERIALS AND METHODS

Study sites and sample trees

The study was conducted at the Iriomote Tropical Forest Tree Breeding Technical Garden at the Forest Tree Breeding Center of the Forestry and Forest Products Research Institute, Okinawa Prefecture, in south-western Japan from September 2010 till February 2012. The numbers of sample clones used for artificial pollination were two *A. mangium* and five *A. auriculiformis* (Table 1). To raise the clones, seeds of both species were bought from CSIRO in 1996 and about 50 seeds of each species from each seed source were sowed in the nursery. After 6 months, about 40 seedlings of each species were planted in the field at intervals of 3 m × 3 m. In 2003, clones consisting of 1 to 3 ramets were established by layering the *A. auriculiformis* and *A. mangium* seedlings that grew normally from each seed source. All layered ramets were potted (black colour, diameter 50 cm and height 45 cm) in a mesh house at intervals of 3 m × 3 m to exclude pollinators. The height of each ramet was maintained at 3 m by pruning for easy observation of flowers.

Artificial pollination

Artificial pollination was conducted from 13 October 2010 till 29 November 2010. Every morning, the condition of flowering of parent clones to be pollinated was confirmed. After determining which flowering clone was the adult male, pollen was collected from the clone using polyvinyl tube (diameter 0.5 cm, length 7 cm) which was inserted into spikes when nearly all the flowers on each spike had bloomed. To attach numerous pollen grains (polyads) in the tube to the inner wall, the tube was moved up and down in the spikes for five to ten times.

Immediately, the tube containing pollen at the inner wall was inserted into another spike which was used as female parent. To provide opportunity for the attachment of as many polyads as possible to the stigma, the tube was again gently moved up and down for five to ten times. The number of artificial pollinations conducted in each cross combination ranged from 17 to 26 (Table 2). Intraspecific pollination in *A. auriculiformis* or *A. mangium* was also conducted as control, each cross combination ranging from 11 to 20 (Table 3). Artificial pollination was performed until noon because pollination was limited to the morning hours (Ogawa et al. 2008). All sample spikes were marked using coloured bin liners for identification of the mating scheme.

Table 1 Sample clones, seed source of the clone, number of ramets examined and location of the seed source for *Acacia mangium* and *A. auriculiformis*

Species	Clone	Seed source	Number of ramets examined	Latitude	Longitude	Altitude (m asl)
A. mangium	512	Queensland	2	18° 14' S	145° 57' E	18
	525	Papua New Guinea	2	08° 37' S	142° 47' E	40
A. auriculifomis	130	Papua New Guinea	2	8° 50' S	141° 38' E	18
	147	Thailand	2	12° 35' N	101° 15' E	0
	148		3			
	149		1			
	151	Queensland	2	12° 34' S	143° 10' E	20

asl = above sea level

Table 2 Number of artificial pollinations in each reciprocal cross combination

A. mangium clone	Male/female (M/F)	*A. auriculiformis* clone									
		130		147		148		149		151	
		F	M	F	M	F	M	F	M	F	M
512	M	20		20		20		20		20	
	F		23		26		22		23		18
525	M	20		20		20		20		20	
	F		17		21		20		20		20

Table 3 Number of artificial pollinations in each control cross combination

Female tree species	Clone	Male tree species and clone				
		A. mangium		*A. auriculiformis*		
		512	525	147	148	149
A. mangium	512		11			
	525	11				
A. auriculiformis	147				20	20
	148			20		20
	149			20	20	

Pollen germination test and number of flowers per spike

Apart from the tubes collected from each clone for artificial pollination, 20 other tubes were used for germination tests. Those tests were conducted one day after the time of pollen collection (Yamaguchi & Ogawa 2008). Pollens were removed from the stored tubes using brush and then more than 300 pollens were dropped onto the surface of a medium consisting of 20% sucrose and 1% agar in a Petri dish. Following incubation at 23 °C for 48 hours, the condition of the polyads comprising 16 pollen grains was examined under a microscope. Polyads with at least one extended pollen tube were considered germinated. The mean germination percentage of pollen grains for *A. mangium* and *A. auriculiformis* was calculated by dividing the number of polyads with extended pollen tube by the number of polyads investigated for each clone. Spikes of both clones that were just about to flower were collected and the number of buds on each spike was counted except one clone each of each species.

Seed collection, germination and propagation

Approximately 6 months after pollination, seeds produced were collected from mature pods. The number of spikes containing seeds and the total number of mature and immature seeds were counted for each cross combination. After excluding immature seeds, mature seeds were treated with hot water (boiling) for 1 min to enhance germination (Poulsen et al. 1998) and then sowed in boxes (50 cm × 30 cm) filled with fertile soil. Germinated seeds were immediately transplanted to pots in the field. Ungerminated seeds were considered dead seeds. Seedlings in the field were watered twice daily until collection of phyllodes.

DNA analysis

One month after the transplantation, a phyllode from each seedling of interspecific pollination was collected and desiccated at 40 °C for 24 hours. DNA was extracted from the treated phyllodes using the cetyltrimethylammonium bromide method. The DNA was then used as template for polymerase chain reactions (PCRs)

designed to amplify the sequence characterised amplified region (SCAR) marker (Huang et al. 2005). Amplified PCR products were analysed by electrophoresis in 1.3% agarose gels containing SYBR Safe stain. From the visualised DNA bands, the percentage of hybrid seedlings generated from each cross combination was calculated. DNA analysis was not conducted on individuals that were dead or whose phyllode did not expand within one month after transplantation.

Statistical analysis

Pollen germination percentage and the number of flowers per spike were compared between clones using ANOVA and, if significant, Tukey's multiple test was conducted to detect the difference between them. The percentage of spike-forming pods, the number of pods formed per spike, the number of whole seeds and mature seeds per spike, the seed germination percentage per spike and the number of seedlings acquired per spike were compared between reciprocal crossings using t-test. Correlations between the pollen germination percentage of each clone and the data concerning the percentage of spike-forming pods, the number of pods formed per spike and the number of whole seeds per spike when the pollen was used were also calculated for each cross combination.

RESULTS

Mean germination percentages of pollen grains ranged from 13.7 to 37.2% for *A. mangium* clones and from 13.9 to 83.9% for *A. auriculiformis* clones, and showed significant difference between clones (one-way ANOVA, $F = 106.2$, $p < 0.01$) (Table 4). From the multiple tests, pollen germination percentage were divided into three groups, namely, low (clones 525 and 148), medium (clones 512 and 147) and high (clones 130, 149 and 151) levels.

Mean number of flowers per spike was 186 on *A. mangium* clone 512 but ranged from 76 to 98 on all *A. auriculiformis* clones. There is significant difference between clones (one-way ANOVA, $F = 73.1$, $p < 0.01$) (Table 5). From the multiple tests,

Table 4 Mean germination percentage of pollen grains (± SE) in each clone of *Acacia mangium* and *A. auriculiformis*

Species	Clone	Germination percentage (Mean ± SE)	F	Result of multiple test[1]
A. mangium	512	37.2 ± 4.1		a
	525	13.7 ± 1.4		b
A. auriculifomis	130	77.4 ± 1.7		c
	147	25.0 ± 2.9	106.2**	ab
	148	13.9 ± 1.9		b
	149	83.8 ± 1.8		c
	151	73.5 ± 5.1		c

**$p < 0.01$, [1]same letters are not significant ($p > 0.05$); SE = standard error

Table 5 Number of flowers per spike in each clone of *Acacia mangium* and *A. auriculiformis*

Species	Clone	Number of flowers (Mean ± SE)	F	Result of multiple test[1]
A. mangium	512	186.3 ± 9.0		a
A. auriculifomis	130	96.4 ± 3.4	73.1**	b
	147	76.0 ± 3.6		b
	148	98.3 ± 4.3		b
	149	96.4 ± 6.7		b

**$p < 0.01$, [1]same letters are not significant ($p > 0.05$); SE = standard error

the number of flowers per spike on *A. mangium* clone 512 is significantly larger than that on all *A. auriculiformis* clones.

Percentage of spike-forming pods ranged from 11.5 to 65.2% (averaging 30.5%) between cross combinations when using an *A. mangium* clone as female parent (Table 6). On the other hand, when *A. auriculiformis* clone was used as female parent, the values ranged from 15 to 85% (average 45.5%). The average percentages are not significantly different from each other ($p > 0.05$). The percentages of spike-forming pods were from 9.1 to 36.4% and from 0 to 50% between cross combinations for intraspecific pollination of *A. mangium* and *A. auriculiformis* respectively (Table 7).

Mean number of pods formed per spike ranged from 0.3 to 3.2 (average 1.9) between cross combinations with *A. mangium* clone as female parent (Table 8). It ranged from 0.6 to 10.0 (average 3.0) when *A. auriculiformis* clone was the female parent. Averages are not significantly different from each other ($p > 0.05$). Mean number of pods formed per spike was from 0.6 to 4.3 and from 0 to 2.1 among cross combinations on the intraspecific pollination of *A. mangium* and *A. auriculiformis* respectively (Table 9).

Mean number of whole seeds and mature seeds produced per spike ranged from 1.5 to 27.6 and from 1.3 to 26.9 between cross combinations, averaging 15.1 and 12.1 respectively when using *A. mangium* clone as the female parent (Table 10). On the other hand, with *A. auriculiformis* clone as female parent the values ranged from 2.0 to 68.3 and from 0 to 10.7 (average 17.6 and 4.1) respectively. The average number of whole seeds produced is not significantly different between them ($p > 0.05$) but the average number of mature seeds produced is significantly larger when *A. mangium* was used as the female parent ($p < 0.05$). The number of whole seeds produced per spike was from 3.9 to 29.8 and from 0 to 7.3 and that of mature seeds from 3.6 to 6.4 and from 0 to 2.5, between cross combinations on the intraspecific pollination of *A. mangium* and *A. auriculiformis*, respectively (Table 11).

Mean seed germination percentage per spike ranged from 14.1 to 61.3% (average 26.8%) between cross combinations using *A. mangium* clone as female parent (Table 12). On the other hand, it ranged from 0 to 55.6% (average 13.6%) between cross combinations when *A. auriculiformis* clone was the female parent. The average percentage is significantly higher when *A. mangium* was used as the female parent ($p < 0.05$). Mean seed germination percentage per spike ranged from 6.4 to 25.0% and from 0 to 50.0% between cross combinations on the intraspecific pollination of *A. mangium* and *A. auriculiformis* respectively (Table 13). Mean number of seedlings acquired per spike ranged from 0.23 to 5.89 (average 3.45) between cross combinations using *A. mangium* clones as the female parent (Table 14). When *A. auriculiformis* clone was used as female parent, values ranged from 0 to 1.05 (average 0.36) between cross combinations. The average number is significantly higher when *A. mangium* was used as female parent ($p < 0.05$). Mean number of seedlings acquired per spike ranged from 0.36 to 0.91 and from 0 to 0.10 between cross combinations on the intraspecific pollination of *A. mangium* and *A. auriculiformis* respectively (Table 15).

All relationships between pollen germination percentage and percentage of spike-forming pods, mean number of pods formed per spike and mean number of whole seeds produced per spike when the pollen was used showed positive correlations, although they are not significant ($r = 0.35$, 0.36 and 0.18 respectively, $p > 0.05$) for each cross combination. Phyllodes used for DNA analysis were collected from 336 seedlings that were generated following pollination by interspecific pollination. However, 10 samples could not be analysed because their DNA could not be amplified. PCR analysis based on the SCAR marker revealed that 323 (99.1%) of these seedlings were hybrids (Figure 1, Table 16), suggesting that self-compatibility seldom occurred in this crossing method. The three seedlings detected as non-hybrid *A. mangium* were obtained from the cross combination using clones 512 and 130.

DISCUSSION

In this study, hybrid seeds could be obtained from all cross combinations with very few contaminations of *A. mangium* seeds. Therefore, unlike results on reciprocal crossings between *Eucalyptus ovata* and *E. globulus* (Lopez et al. 2000), a reciprocal crossing of artificial pollination between *A. auriculiformis* and *A. mangium* can be

Table 6 Percentage of spike-forming pods in each reciprocal cross combination for *Acacia mangium* and *A. auriculiformis*

A. mangium clone	Male/ female (M/F)	*A. auriculiformis* clone										Mean % ± SE	Mean % ± SE (*A. mangium* as female parents)	Mean % ± SE (*A. auriculiformis* as female parents)
		130		147		148		149		151				
		F	M	F	M	F	M	F	M	F	M			
512	M	85.0		25.0		60.0		25.0		45.0		48.0 ± 11.4		
	F		65.2		11.5		13.6		34.8		16.7	28.4 ± 10.1	30.5 ± 5.2	45.5 ± 7.7
525	M	40.0		40.0		15.0		35.0		85.0		43.0 ± 11.5		
	F		35.3		42.9		25.0		20.0		40.0	32.6 ± 4.4		

SE = standard error

Table 7 Percentage of spike-forming pods in each control cross combination for *Acacia mangium* and *A. auriculiformis*

Female tree species	Clone	Male tree species and clone					Mean % ± SE
		A. mangium clone		*A. auriculiformis* clone			
		512	525	147	148	149	
A. mangium	512		36.4				36.4*
	525	9.1					9.1*
A. auriculiformis	147				10.0	0.0	5.0 ± 5.0
	148			50.0		30.0	40.0 ± 10.0
	149			20.0	25.0		22.5 ± 2.5

*SE = standard error; there was only one cross combination, thus, no SE was calculated

Table 8 Mean number of pods formed per spike (± SE) in each reciprocal cross combination for *Acacia mangium* and *A. auriculiformis*

A. mangium clone	Male/ female (M/F)	*A. auriculiformis* clone										Mean ± SE
		130		147		148		149		151		
		F	M	F	M	F	M	F	M	F	M	
512	M	6.3 ± 1.6		0.8 ± 0.3		4.3 ± 1.2		0.6 ± 0.3		2.9 ± 1.1		3.0 ± 1.1
	F		3.0 ± 0.8		0.3 ± 0.2		0.4 ± 0.2		2.7 ± 1.1		2.4 ± 1.6	1.8 ± 0.6
525	M	2.5 ± 0.8		0.9 ± 0.3		0.7 ± 0.5		0.9 ± 0.4		10.0 ± 2.2		3.0 ± 1.8
	F		1.5 ± 0.6		3.2 ± 1.1		0.9 ± 0.4		2.2 ± 1.1		2.0 ± 0.8	2.0 ± 0.4

SE = standard error

Table 9 Mean number of pods formed per spike in each control pollination of *Acacia mangium* and *A. auriculiformis*

Female tree species	Clone	Male tree species and clone					Mean ± SE
		A. mangium clone		*A. auriculiformis* clone			
		512	525	147	148	149	
A. mangium	512		4.3 ± 2.1				4.3 ± 2.1
	525	0.6 ± 0.6					0.6 ± 0.6
A. auriculiformis	147				0.2 ± 0.2	0	0.1 ± 0.1
	148			2.1 ± 0.8		0.8 ± 0.3	1.5 ± 0.7
	149			0.4 ± 0.2	0.8 ± 0.3		0.6 ± 0.2

SE = standard error

Table 10 Mean number of seeds produced per spike (± SE) in each reciprocal cross combination for *Acacia mangium* and *A. auriculiformis*

A. mangium clone	Male/female (M/F)	*A. auriculiformis* clone										Mean ± SE	Mean ± SE (*A. mangium* as female parents)	Mean ± SE (*A. auriculiformis* as female parents)
		130		147		148		149		151				
		F	M	F	M	F	M	F	M	F	M			
512	M	27.6 ± 7.5 (10.4, 17.2)		3.4 ± 2.3 (0.6, 2.8)		22.5 ± 7.1 (0.3, 22.3)		4.6 ± 2.7 (1.4, 3.2)		21.9 ± 7.5 (7.2, 14.7)		16.0 ± 5.0 (4.0 ± 2.0, 12.0 ± 3.9)		
	F		23.4 ± 5.8 (17.5, 5.9)		1.5 ± 1.5 (1.3, 0.2)		2.8 ± 1.7 (2.1, 0.7)		13.0 ± 4.8 (6.3, 6.7)		16.3 ± 11.3 (9.6, 6.7)	11.4 ± 4.1 (7.4 ± 2.9, 4.0 ± 1.5)	15.1 ± 3.7	17.6 ± 8.8
525	M	15.2 ± 5.1 (9.0, 6.2)		2.0 ± 0.9 (0.3, 1.7)		7.6 ± 3.0 (0.0, 7.6)		3.3 ± 2.9 (0.6, 2.7)		68.3 ± 16.1 (10.7, 57.6)		19.3 ± 12.5 (4.1 ± 2.4, 15.2 ± 10.7)	(12.1 ± 3.1, 3.0 ± 1.1)	(4.1 ± 2.2, 13.6 ± 7.3)
	F		27.6 ± 4.7 (26.9, 0.7)		19.2 ± 8.1 (15.4, 3.8)		6.9 ± 3.2 (6.5, 0.4)		18.9 ± 9.8 (16.5, 2.4)		20.9 ± 8.8 (18.5, 2.4)	18.7 ± 3.3 (16.8 ± 3.3, 1.9 ± 0.6)		

Valume in parentheses indicate mature seeds and immature seeds respectively

Table 11 Mean number of seeds produced per spike (mean ± SE) in each control cross combination for *Acacia mangium* and *A. auriculiformis*

Female tree species	Clone	Male tree species and clone					Mean ± SE
		A. mangium clone		*A. auriculiformis* clone			
		512	525	147	148	149	
A. mangium	512		29.8 ± 15.7 (6.4, 23.4)				29.8 ± 15.7 (6.4, 23.4)
	525	3.9 ± 3.9 (3.6, 0.3)					3.9 ± 3.9 (3.6, 0.3)
A. auriculifomis	147				0.7 ± 0.6 (0.2, 0.5)	0.0 (0.0, 0.0)	0.35 ± 0.35 (0.1 ± 0.1, 0.3 ± 0.3)
	148			7.3 ± 2.6 (0.3, 7.0)		4.0 ± 1.9 (0.4, 3.6)	5.7 ± 1.7 (0.4 ± 0.1, 5.3 ± 1.7)
	149			2.2 ± 1.2 (0.7, 1.5)	5.7 ± 2.8 (2.5, 3.2)		4.0 ± 1.8 (1.6 ± 0.9, 2.4 ± 0.9)

Valume in parentheses indicate mature seeds and immature seeds respectively; SE = standard error

Table 12 Mean germination percentage of seeds per spike (± SE) in each reciprocal cross combination for *Acacia mangium* and *A. auriculiformis*

A. mangium clone	Male/female (M/F)	*A. auriculiformis* clone										Mean % ± SE
		130		147		148		149		151		
		F	M	F	M	F	M	F	M	F	M	
512	M	0.4 ± 0.3		0.0		0.0		52.2 ± 21.2		17.7 ± 10.5		14.1 ± 10.1
	F		16.3 ± 5.6		29.6 ± 17.3		17.1 ± 13.1		25.2 ± 11.1		61.3	29.9 ± 8.2
525	M	14.1 ± 6.4		16.7 ± 16.7				19.7 ± 12.2		1.3 ± 0.9		13.0 ± 4.0
	F		26.6 ± 10.1		20.3 ± 4.8		32.8 ± 11.8		24.9 ± 11.4		14.1 ± 6.4	23.7 ± 3.1

SE = standard error

Table 13 Mean germination percentage of seeds per spike (± SE) in each control cross combination for *Acacia mangium* and *A. auriculiformis*

Female tree species	Clone	Male tree species and clone					Mean % ± SE
		A. mangium clone		*A. auriculiformis* clone			
		512	525	147	148	149	
A. mangium	512		6.4 ± 4.7				6.4
	525	25.0					25.0
A. auriculiformis	147				50.0		50.0
	148			0.0		0.0	0.0
	149			0.0	2.9 ± 2.9		1.5 ± 1.5

SE = standard error

Table 14 Mean number of seedlings per spike (mean ± SE) in each reciprocal cross combination for *Acacia mangium* and *A. auriculiformis*

A. mangium clone	Male/ female (M/F)	*A. auriculiformis* clone										Mean % ± SE
		130		147		148		149		151		
		F	M	F	M	F	M	F	M	F	M	
512	M	0.15 ± 0.11		0.00		0.00		0.75 ± 0.41		1.05 ± 0.81		0.39 ± 0.22
	F		4.65 ± 1.96		0.65 ± 0.58		0.23 ± 0.16		2.09 ± 1.01		5.89 ± 5.89	2.70 ± 1.11
525	M	1.05 ± 0.60		0.05 ± 0.05		0.00		0.20 ± 0.14		0.30 ± 0.18		0.32 ± 0.19
	F		3.53 ± 1.80		5.00 ± 1.90		2.85 ± 1.52		5.05 ± 3.85		4.55 ± 3.05	4.20 ± 0.43

SE = standard error

Table 15 Mean number of seedlings per spike (± SE) in each control cross combination for *Acacia mangium* and *A. auriculiformis*

Female tree species	Clone	Male tree species and clone					Mean % ± SE
		A. mangium clone		*A. auriculiformis* clone			
		512	525	147	148	149	
A. mangium	512		0.36 ± 0.28				0.36
	525	0.91 ± 0.91					0.91
A. auriculiformis	147				0.10 ± 0.10	0.00	0.05 ± 0.05
	148			0.00		0.00	0.00
	149			0.00	0.05 ± 0.05		0.03 ± 0.03

SE = standard error

Table 16 Proportion of seedlings (%) recognised as hybrids by DNA analysis for each cross combination for *Acacia mangium* and *A. auriculiformis*

A. mangium clone	Male/ female (M/F)	*A. auriculiformis* clone									
		130		147		148		149		151	
		F	M	F	M	F	M	F	M	F	M
512	M							100 (9)		100 (7)	
	F		92.3 (43)		100 (14)		100 (2)		100 (41)		100 (19)
525	M	100 (18)		100 (1)				100 (2)		100 (3)	
	F		100 (36)		100 (38)		100 (21)		100 (48)		100 (34)

Valume in parentheses indicate number of samples

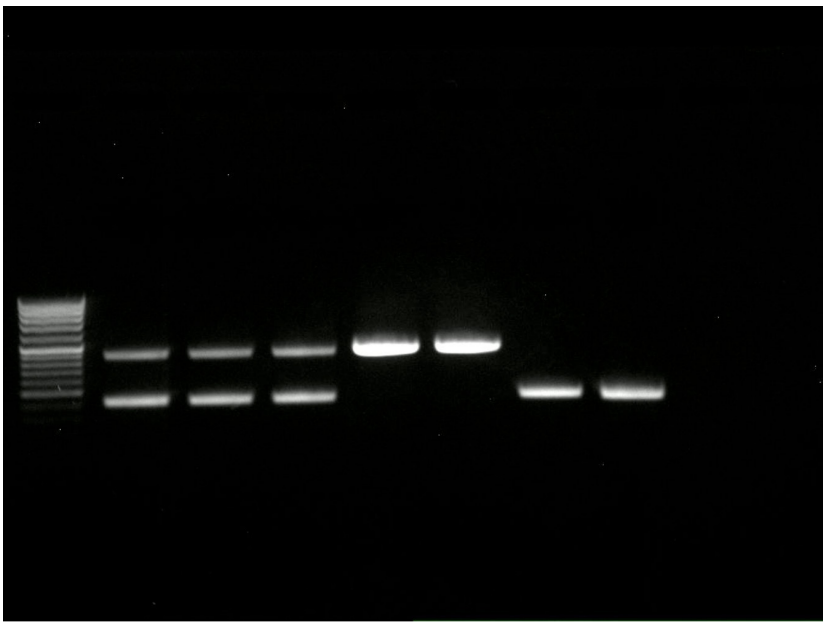

Figure 1 PCR analysis on the SCAR marker; lane 1 shows the size of marker, lanes 2–4 show the hybrids, lanes 5 and 6 show *Acacia mangium* and lanes 7 and 8 show *A. auriculiformis*

conducted for producing hybrid seeds. In this study, some considerable results were obtained. Firstly, the ability of pollen grains to germinate on each clone could be divided into three group regardless of species but this difference did not substantially affect the percentage of spike-forming pods, the number of pods formed per spike, or the number of seeds produced per spike (Table 4). In *Acacia podalyriifolia*, pollen germination percentage on the stigma is higher than that in agar germination test (Beck-Pay 2012). Therefore, the actual germination percentages on *A. mangium* and *A. auriculiformis* stigmas in this study could actually be higher than those on agar medium. This suggests that a sufficient number of hybrid seeds can be obtained even if the germination percentage on the medium is low, such as in clones 525 and 148.

Although the number of flowers per spike for *A. mangium* was almost double that on *A. auriculiformis* (Table 5), there is no significant difference in the percentage of spike-forming pods, the number of pods formed per spike and the number of seeds produced per spike (Tables 6–11). This suggests that the percentage of pollens taking on stigmas or the percentage of pollen tubes reaching ovules was greater in *A. mangium* pollens. There appears to be weak attraction by *A. mangium* ovules for *A. auriculiformis* pollen tubes (Sedgley et al. 1992a). This caused a greater number of mature seeds to be produced when *A. auriculiformis* was used as female parent. Therefore, this weak attraction was the reason for lack of significant difference in percentage of spike-forming pods, number of pods formed per spike and number of seeds produced per spike between the trees. Thus, difference in the number of flowers per spike did not affect the efficiency of artificial pollination between reciprocal crossings.

In this study, although the number of whole seeds produced is not significantly different between reciprocal crossings, average number of mature seeds produced is significantly larger when *A. mangium* was used as female parent (Tables 10 and 11), indicating that the maturation of seeds was not very successful when *A. auriculiformis* was used as female parent. In interspecific pollination of *Magnolia stellate* and *M. salicifolia*, there is a stunting of seeds when *M. stellate* is used as female adults (Tanihaya et al. 2014). Hybridisation and setting of seeds are more successful when the parent of the higher ploidy level serves as seed parent (Hauser et al. 1998). In this study, the ploidy level of two species was the same so that either species can be used for the seed parent. It is interesting that *A. auriculiformis* clones 147, 148, and 149, which produced low numbers of mature seeds, originated from a seed source in Thailand (Table 1). *Acacia auriculiformis* has higher genetic

differentiation between populations than *A. mangium* (Wickneswari & Norwati 1993). On *Lotus scoparius*, failure of crosses (seeds/flower × seedlings/seed) increases with increasing genetic distance between populations (Montalvo & Ellstrand 2001). Negative heterosis occurrs on F1 hybrids of *Silene alba* when the genetic distance between parental populations is high (Keller et al. 2000). Therefore, it is probable that a barrier on the maturation of seeds may occur in specific population of *A. auriculiformis* as female adults when the genetic distance from *A. mangium* is high (Widyatmoko et al. 2010).

Buds do not form on any spikes when *A. auriculiformis* tree is used as female adult, although the percentage of flowers with penetrated ovules is 50% (Sedgley et al. 1992b). The number of produced seeds is lower in intraspecific pollination of *A. auriculiformis* than interspecific pollination of *A. auriculiformis* and *A. mangium*, suggesting that fecundity as female parents on some *A. auriculiformis* trees may be inferior regardless of tree species as male parents (Sedgley et al. 1992a, Kato et al. 2012). In this study, the percentage of spike-forming pods, the number of pods formed per spike, the number of seeds produced per spike, germination percentage of seeds per spike and number of seedlings per spike were also low in the intraspecific pollination of *A. auriculiformis* compared with the reciprocal crossings and the intraspecific pollination of *A. mangium* (Tables 6–15). This suggests that *A. auriculiformis* clones 147, 148 and 149 are inferior in fecundity as female adults.

It has been reported that 2.6% of harvested mature seeds obtained from interspecific pollination (*A. auriculiformis* × *A. mangium*) by tube pollination are recognised as *A. auriculiformis* (Kato et al. 2012). In this study, as a result of interspecific artificial pollination, 0.9% of seedlings were not hybrid and recognised as *A. mangium* (Figure 1 and Table 16). These suggest that self-compatibility occurred on both tree species by tube pollination. Self-compatibility occurs in only one of four trees (Kato et al. 2012). In this study, self-compatibility occurred in only one combination of interspecific pollination. These also suggest that self-pollination may occur in specific trees in each species.

Seed germination percentage per spike in this study is low compared with that of other reports (Sedgley et al. 1992a, Kato et al. 2012). Percentage was 0% on some *A. auriculiformis* clones, resulting in very low acquisition of seedlings (Tables 12–15). In this study, seeds of both species were pretreated at 100 °C for 1 min but Khomane and Bhosale (2002) reported that the best pretreatment for germination of *A. mangium* is 70 °C for 15 min. Exposure to 100 °C for 10 min is the best pretreatment for germination of *A. auriculiformis* (Sharma et al. 2008). These suggest that the condition of pretreatment may vary with species and clones. Therefore, even if the same number of mature seeds is produced between reciprocal crossings, the number of seedlings acquired may be influenced by pretreatment for germination, indicating that detection of optimal pretreatment for germination beforehand for each species or clones is needed.

Accordingly, in this study, three points are suggested with regard to the breeding programme for establishing *Acacia* hybrid: (1) in general, each reciprocal crossing can be done because the number of seeds produced per spike is not different between the crossings, (2) because of high genetic diversity and low fecundity in some *A. auriculiformis* trees, reproductive traits of the trees must be investigated precisely for using interspecific pollination and (3) since self-compatibility may occur in specific trees on both species by tube pollination, it is recommended to manipulate emasculation of spikes if only hybrid seeds are harvested by interspecific pollination.

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