Karyotypic Comparison of Acacia mangium Willd., A. auriculiformis A. Cunn. Ex Benth and Their F_1 and F_2 Hybrids

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Summary

Cytological data for *Acacia mangium* Willd., *A. auriculiformis* A. Cunn. Ex Benth and their F_1 and F_2 hybrids are presented. Somatic chromosome number of there species confirmed that they have 2n=2x=26. Average absolute chromosome length ranged from 1.210 μm ± 0.0010 μm in *A. auriculiformis* to 2.467 μm ± 0.0278 μm in *A. mangium*, with hybrids F_1 and F_2 producing intermediate values. These karyotypes are unique and species specific.

Key words: Acacia auriculiformis, A. mangium, F_1 hybrid, F_2 hybrid, karyotype, chromosome number.

FDC: 168; 165.42; 165.7; 176.1 *Acacia auriculiformis*; 176.1 *Acacia mangium.*

Introduction

The genus *Acacia,* comprising more than 500 species of shrubs and trees, is very well distributed in the tropics and sub-tropics. Tropical acacias have shown potentials as multipurpose species. They are grown for wood production, soil improvement and conservation, fire breaks and in agroforestry projects. Acacias also adapt well on soils in the humid tropics (Turnbull, 1986). *Acacia mangium* Willd. and *A. auriculiformis* Cunn. ex Benth are native to Australia, Papua New Guinea and Indonesia and are commonly planted species.

In Malaysia, *A. mangium* is widely used as a forest plantation species. Unfortunately, *A. mangium* trees grown in Sabah (1 of 2 states of East Malaysia) have originated from a single half-sib family. Progeny trials of several successive generations have indicated steady decline in vigour (Plant, 1981; Sim, 1986) probably due to inbreeding depression. There is urgent need for gene pool expansion in *A. mangium* plantations to redeem favourable heterozygous genotypes (Plant, 1981).

Steps have been taken to broaden the genetic base of the *A. mangium* through various tree improvement program. One approach has been to produce hybrids between *A. mangium* x *A. auriculiformis* for operational planting in Sabah (Sim, 1986). These hybrids possess desirable characteristics such as comparable growth performance to the parent species, possible heterosis (hybrid vigour), intermediate form characteristics, better wood utilization properties, and possibly disease resistance (Pinso and Nasi, 1991). Additionally, the hybrids have potential as a multipurpose fast-growing hardwood for reforestation in tropical countries (Rufelds, 1988).

Identification of hybrid from parental trees based from their morphology is rather difficult. Taxonomic classification, within the genus, based on external morphological characteristics, is complicated by considerable genetic variation and varying environmental factors. This study will determine if karyotypic variation in *A. mangium, A. auriculiformis* and their F_1 and F_2 hybrids based on chromosome number and size (length) at metaphase can be used for hybrid identification.

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Materials and Methods

Meristematic cells were examined in rapidly growing root tips of potted seedlings of *A. auriculiformis, A. mangium,* F_1 hybrids (*A. mangium* x *A. auriculiformis*) and F_2 hybrid. Each species and hybrid generation was represented by 6 healthy seedlings that were selected randomly. The seedlot number for the parental trees of *A. auriculiformis* and *A. mangium* are 2555 and 2532 respectively, both originated from provenance Lungmanis, Sabah. The seeds were provided by the Forest Research Institute, Sepilok, Sabah, Malaysia. The F_1 and F_2 seedlings have been selected randomly. New root growth was stimulated by removing the lower 2 centimeters of the root ball, and filling this area with moss.

The root tips were pretreated in 0.002 M 1-Bromonaphalene and were then fixed in Carnoy's fixative (6:3:1 absolute alcohol, acetic acid and chloroform) for overnight in a refrigerator. The fixed root tips were hydrolysed in warm 1N HCl for 35 minutes at 60 °C and stained in Feulgen for 3 hours. Squash preparations were made in diluted 1 % acetocarmine.

Cells with well spread chromosomes were photographed for the karyotype analyses. The total length of each chromosome from the 6 good cells of each species and hybrid generation were measured using a micrometer scale. These parameters were assessed statistically using an unpaired t test with significant levels of $p<0.05$.

Results

A summary on the measurements of chromosome number and length of *A. auriculiformis, A. mangium* and their F_1 and F_2 hybrids are shown in *table 1.* The somatic chromosome number determination in these species and their hybrids showed a diploid number of 2n=2x=26 (*Tab. 1, Figs. 1* and *2*) thus confirming the result of past studies on Australian acacias. Generally, the centromeric positions in the smaller chromosomes were difficult to distinguish.

Table 1 shows the relative sizes (length) of *A. auriculiformis, A. mangium,* F_1 hybrid and F_2 hybrid ranging from 0.88 μm to 1.58 μm, 1.28 μm to 4.26 μm, 0.88 μm to 2.10 μm and 0.88 μm to 2.70 μm respectively. The ranges of absolute size of the *A. auriculiformis* complement are smaller than *A. mangium* whereas the absolute sizes of both hybrids were found to be intermediate between those of their parents.

The unpaired 't' test of average chromosome length in 6 cells was found to be significant different ($p<0.05$) between *A. auriculiformis* and *A. mangium,* and between the F_1 hybrids to their parents (*Tab. 1*). Significant difference ($p<0.05$) was also found between F_2 hybrid and their parents. However the average chromosome length

Table 1. — Results of chromosome analysis of *Acacia* species and their hybrids based on 6 cells sample.

	Somatic number	Range of chromosome length (μm)	Average chromosome Length ± SE	Significant Differences Between Species	"t" Average Chromosome Length
A. auriculiformis	2n = 26	0.88 - 1.58	1.210 ± 0.0010	*A. auriculiformis* - *A. mangium*	7.4069*
				A. auriculiformis - F_1 hybrid	5.1235*
A. mangium	2n = 26	1.28 - 4.26	2.467 ± 0.0278	*A. auriculiformis* - F_2 hybrid	2.7107*
				A. mangium - F_1 hybrid	5.8872*
F_1 hybrid	2n = 26	0.88 - 2.10	1.461 ± 0.0014	A. mangium - F_2 hybrid	3.3480*
				F_1 hybrid - F_2 hybrid	1.2360ns
F_2 hybrid	2n = 26	0.88 - 2.70	1.674 ± 0.0283		

*) Significantly different at $p < 0.05$
ns: no significant difference
SE; standard error

was not significantly different between, the F_1 and F_2 hybrid generations.

Discussion and Conclusion

The karyotype analyses revealed a lack of variation in the somatic cromosome number among the parental species and hybrids. This result is similar to the findings of MUHAMMAD (1951) on Australian species of *Acacia decurrens*, *A. mollissima* and their F_1 and F_2 hybrids. He also reported that the Australian acacias possess longer chromosomes than the African acacias with the chromosome length of more than 3 μm in the former and 1 μm in the latter species.

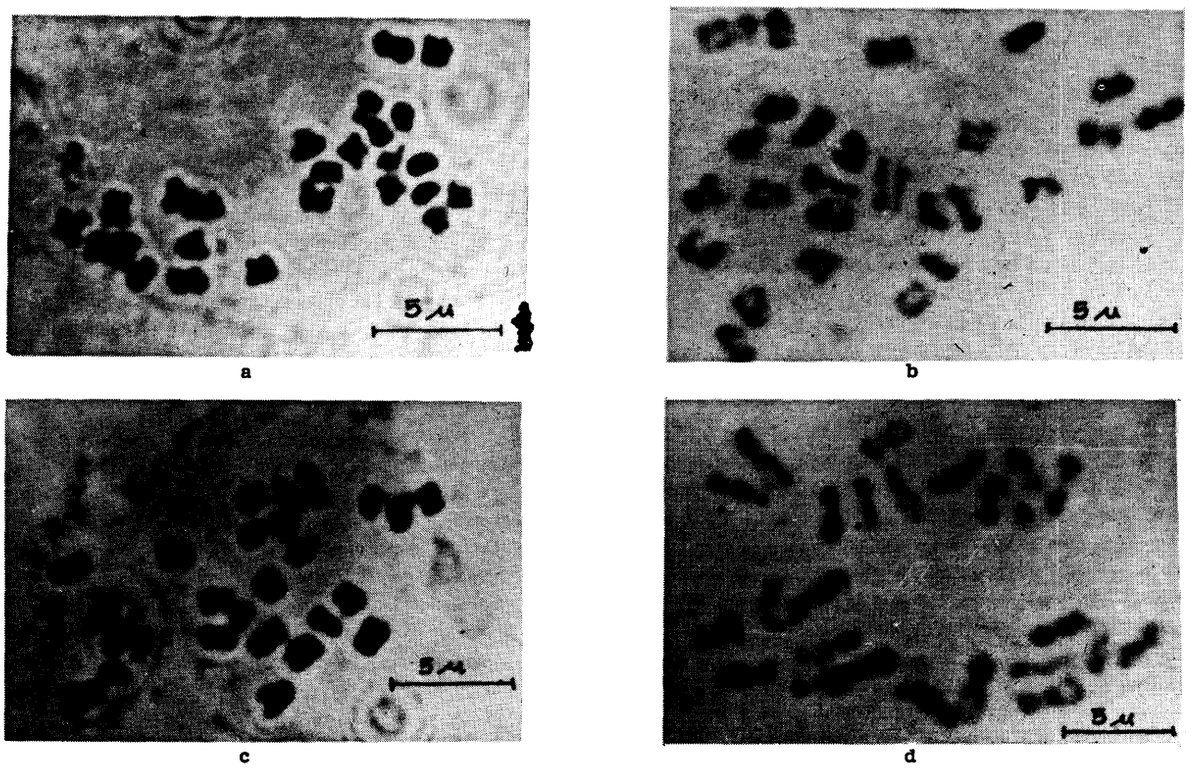

Figure 1. — Mitotic metaphase in root tips of a) *A. auriculiformis*, b) F_1 hybrid, c) F_2 hybrid and d) *A.* mangium, showing 2 n = 26

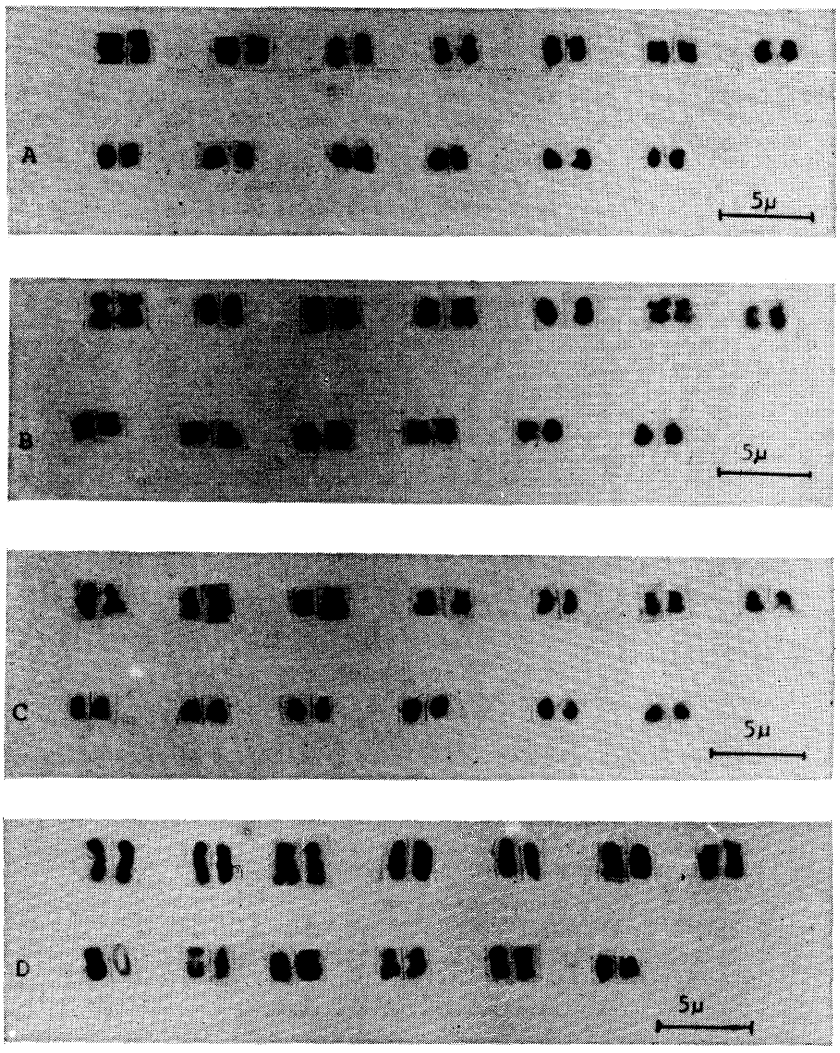

Figure 2. — Karyotypes of *Acacia auriculiformis* (A), *A. mangium* (D) and their F_1 (B) and F_2 (C) hybrids, all showing 13 pairs of chromosomes or somatic chromosome number of 2n = 26. The scale shows 5 μm.

However, no triploids (2n = 3x = 39) were detected as found in a natural *A. senegal* x *A. mellifera* hybrid swarm in Africa (MUHAMMAD, 1951).

The absolute size differences between both *A. auriculiformis* and *A. mangium* and also between their F_1 and F_2 hybrids deduced in this investigation may prove to be an effective method for taxonomic identification where morphological variations are limited. These interspecific differences were also observed in karyotypic comparisons between other Australian Acacia species and their hybrids by MUHAMMAD (1951). The absolute size difference between the parent species and their hybrids have been inferred to be due to the genotypic factor than environmental factor of these species and their hybrids (MUHAMMAD, 1951). Similar results were observed in terms of chromosome size in karyotypic comparison between 4 species of Crepis where their karyotypic relationship is paralleled by comparable variation in the size of florets and achenes (viz SWANSON et al., 1988). There was no intraspecific variation between karyotypes of the F_1 and F_2 hybrids. Therefore, karyotype analysis cannot be used to separate hybrid generations.

The detailed comparison of the karyotype between *Acacia* species and their hybrids has not been made on the basis of the position of the centromere as the centromeres in some of the chromosomes were not very clear. Due to this shortcoming, thus karyotypic analyses in this study have been based on the measured chromosome length. This limitation is possibly due in part, to the unsatisfactory staining of chromosomes in the root tip squashes. MUHAMMAD (1951) hypothesized out that the high tannin content in the root cells of Acacia spp could cause unsatisfactory staining.

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Pollen Viability and Seed Set of Silver Fir (Abies alba Mill.) in Polluted Areas of Slovakia

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Abstract

Viability parameters of pollen grains collected from 16 trees of silver fir (*Abies alba* MILL.) growing in 3 locations in Slovakia under varying degrees of air pollution were compared. In vitro pollen germination tests were compared with controlled crossing experiments. The percentage of germinating pollen grains and pollen tube length varied considerably between individual trees. Both these characteristics were highest in samples from a relatively unpolluted locality (Jedlove Kostolany). Germination of pollen samples from a moderately polluted locality (Repiste) was reduced by 20 %, and in samples from highly polluted habitats (Mociar and Kamenec) germination was reduced by 76 % and 84 %, respectively. In 3 samples of 9 trees from the highly polluted locations, the pollen completely failed to germinate in vitro. Pollen tube length was reduced by 21 %, 15 % and 33 % of the control mean pollen tubes length in samples from the unpolluted site. Except for 2 maternal trees of silver fir which exhibited an inverse relationship, a positive correlation was observed between the in vitro viability parameters of pollen grains and the amount of filled seeds in trees used in artificial crossing experiments.

Key words: *Abies alba*, pollution, pollen viability, controlled crossing, seed set.

FDC: 181.52; 425.1; 174.7 *Abies alba*; (437).

Introduction

The detrimental effect of air pollution on vegetative parts of plants is a well known and a broadly documented phenomenon. It predominantly refers to changes in leaves at the biochemical, macroscopic and microscopic levels leading to the death of individuals and entire communities (WOLTERS and MARTENS, 1987). In addition, though not so conspicuous, are the effects of air pollution on the reproductive processes in plants. All stages of the reproductive cycle have been shown to be susceptible to air pollutants (SMITH, 1981). At the gametophyte level, it is usually pollen production and viability that are often affected, resulting in a lowered seed set or even a complete absence of seed. Both the direct effect and indirect influence of air pullutants on pollen is believed to be implicated in such cases.

Chronic exposure to high levels of contaminants has resulted in reduction of both pollen viability and number of seeds per cone in *Pinus strobus* and *Pinus resinosa* (HOUSTON and DOCHINGER, 1977) as well as in the reduced size of pollen grains in *Pinus sylvestris* (SHKARLET, 1972; FEDOROV et al., 1983). The indirect effects of pollutants on the microgametophyte are believed to be mediated by changes in the composition of the stigmatic apparatus of female flowers by modifying the biological interaction between pollen and the stigmatic surface (WOLTERS and MARTENS, 1987). Regardless of the way in which the air contaminants affect pollen, the genetic consequence of contamination is a change in competition between the pollen grains on the style resulting in reduction the genetic variation of the next generation (COX, 1984). In spite of this, many antropogenic air pollutants have existed