

STUDIES ON NATURAL HYBRIDS OF *ACACIA MANGIUM* AND *A. AURICULIFORMIS* IN VIETNAM

Le Dinh Kha

Research Centre for Forest Tree Improvement, Forest Science Institute of Vietnam, Chem, Tu Liem, Hanoi, Vietnam

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LE DINH KHA. 2000. Studies on natural hybrids of *Acacia mangium* and *A. auriculiformis* in Vietnam. Natural hybrids of *Acacia mangium* and *A. auriculiformis* have been found in *A. mangium* plantations in Vietnam. Hybrids found at Ba Vi are derived from the Daintree (Queensland) provenance of *A. mangium* and the Darwin (Northern Territory) provenance of *A. auriculiformis*. F₁ hybrid trees in Vietnam have morphological characteristics intermediate between those of the two parent species, and marked hybrid vigour in growth. At 2.5–3 y, the hybrids produced 100–600% greater stem volume than *A. mangium* and *A. auriculiformis*. At 4.5 y of age the hybrids produced, on average, twice the stem volume of *A. mangium*. Wood density of the hybrid is intermediate between the values of the two parents. Paper pulp productivity, and the breaking strength, folding endurance and brightness of paper produced from the wood of the hybrid are superior to those of the parent species. Plus trees were selected from among the F₁ hybrids from Ba Vi, and cutting propagation and clonal tests of these individuals were established. At age 2y, selected hybrid clones displayed markedly better growth than cuttings and seedlings of the two parent species. Integrated assessment of growth, stem straightness, branch size and tree health were used to select the five best hybrid clones, one of which is outstanding for growth and stem form. The five best clones have been propagated by tissue culture of meristems, and are being tested in trial programmes in the main ecological regions of Vietnam.

Key words: *Acacia* hybrids - growth - pulp - clonal tests - propagation

LE DINH KHA. 2000. Kajian terhadap hibrid semula jadi *Acacia mangium* dan *A. auriculiformis* di Vietnam. Hibrid semula jadi *Acacia mangium* dan *A. auriculiformis* ditemui di ladang *A. mangium* di Vietnam. Hibrid yang didapati di Ba Vi diperoleh dari provenans *A. mangium* di Daintree (Queensland) dan provenans Darwin (Daerah Utara) bagi *A. auriculiformis*. Pokok hibrid F₁ di Vietnam mempunyai ciri-ciri morfologi antara ciri-ciri kedua-dua spesies induk, dan menandakan kecergasan pertumbuhan hibrid. Pada umur 2.5 - 3 tahun, hibrid menghasilkan 100 - 600 % isipadu batang yang lebih besar daripada *A. mangium* dan *A. auriculiformis*. Pada umur 4.5 tahun hibrid menghasilkan secara purata dua kali ganda isipadu batang bagi *A. mangium*. Kepadatan kayu bagi hibrid adalah pertengahan antara kedua-dua induk. Produktiviti palpa kertas, kekuatan memecah, ketahanan lipatan dan kecerahan kertas yang dihasilkan daripada kayu hibrid tersebut adalah lebih baik daripada kertas daripada spesies induk. Pokok terbaik yang dipilih daripada hibrid F₁ dari Ba Vi, dan pembiakan tampang dan ujian klon bagi setiap pokok ini telah ditubuhkan. Pada umur 2 tahun, klon hibrid yang terpilih mempamerkan dengan jelasnya pertumbuhan yang lebih baik daripada keratan dan anak benih kedua-dua spesies induk. Penilaian bersepadu

bagi pertumbuhan, kelurusan batang, saiz dahan dan kesihatan pokok digunakan untuk memilih lima klon hibrid yang terbaik, iaitu yang terbaik dari segi pertumbuhan dan bentuk batang. Lima klon terbaik tersebut dibiak oleh kultur tisú meristem, dan sedang diuji dalam program-program percubaan di kawasan ekologi utama.

Introduction

Natural hybrids of *A. mangium* and *A. auriculiformis* were first recorded by Hepburn and Shim (cited in Pinso & Nasi 1991) in Sabah, Malaysia, in 1972. In 1978, Pedley determined a sample from one of these trees to be a hybrid of the two parent species (Pinso & Nasi 1991). The 9-y-old hybrid has been found to have better sulphate pulp and paper properties than the parents (Yamada *et al.* 1992).

Investigation on seedling morphology carried out by Rufelds (1988), and Gan and Sim (1991) showed that hybrid seedlings formed their phyllodes earlier than *A. mangium* but later than *A. auriculiformis*. Their intermediate characters were also observed for other features such as spikes, florets and seeds (Bowen 1981) as well as peroxidase isozyme (Kiang *et al.* 1988) and chromosome morphology (Nor Aini *et al.* 1994). Besides, the isozyme phenotype of natural hybrids is also close to that of *A. auriculiformis* (Sedley & Harbard 1992). In some hybrid individuals, heterosis is obvious and the natural hybrid is very much taller and has much greater stem volume than the pure species (Pinso & Nasi 1991). However, hybrid vigour is not always apparent (Rufelds 1987). The hybrids have fine branching and a tendency for a strong apical dominance which will eventually lead to a tree with single stem and good length of clear bole (Pinyopusarerk 1990). Natural hybrid trees have been vegetatively propagated by cuttings (Griffin 1988) or tissue culture (Darus 1991). Natural hybrid trees have been found in *A. mangium* plantations in all three parts of Vietnam (North, Central and South). This paper describes studies on the hybrid in comparison with the parent species, carried out in Vietnam over the last six years.

Materials and methods

Research on growth performance of acacia hybrids was carried out in Song May (Dong Nai province, South Vietnam; mean annual rainfall 2200 mm, mean annual temperature 26°C) and BaVi (HaTay province, North Vietnam; mean annual rainfall 1300 mm, mean annual temperature 21 °C). Natural acacia hybrids found in Ba Vi are considered to be derived from hybridisation between the Daintree provenance (Queensland, CSIRO seedlot No. 13279) of *A. mangium* and the Darwin provenance (Northern Territory, CSIRO seedlot No. 13191) of *A. auriculiformis*. These hybrids were found at a frequency of 2–3% in plantations established using seed collected from a stand of Daintree provenance *A. mangium* which was growing at Ba Vi, adjacent to a stand of Darwin provenance *A. auriculiformis* with no other *A. auriculiformis* nearby. Acacia hybrids and the two parent species growing in second-generation *A. mangium* plantations at Ba Vi were examined for morphology and growth rate (Le Dinh Kha *et al.* 1993) and paper pulp potential (Le Dinh Kha & Le Quang Phuc 1995).

Thirty hybrid individuals with superior vigour and stem form from plantations at Ba Vi were selected and pollarded at a height of 0.9 m. These individuals were vegetatively propagated from the resprout cuttings induced by the pollarding. The cuttings were used as planting materials in clonal tests (Le Dinh Kha *et al.* 1995). Tested clones were also successfully propagated by tissue culture (Nguyen Ngoc Tan *et al.* 1996).

Studies on paper pulp potential were conducted by the Institute for Cellulose and Paper Industry. Wood samples were collected from 4-y-old trees growing at Ba Vi. Three trees each of the hybrid, *A. auriculiformis* and *A. mangium* were felled for sample collection. The bark of the sampled trees was removed and three stem sections, each 1 m long, were taken from the base, middle and top of each stem. The top section had an upper diameter of 5 cm, and the middle section was located half-way between the top and base sections. For each species, the nine stem sections thus obtained were combined into a single bulk sample for study of pulping and chemical properties. Chemical composition was determined using the group analysing diagram method, so that the sum of substances may be higher than 100% (Nikitin 1960). Paper pulp potential was determined by the sulphate method and hypochlorite was used for bleaching of pulp. Mechanical properties of paper were determined according to ISO standards. Breaking strength is expressed as length measured at tearing point of a standardised paper strip (70 g m⁻² and 15 cm wide) vertically pulled.

Clonal trials of the selected clones were planted at Ba Vi. Pure-species controls were included in the trials. Provenances of *A. mangium* (Pongaki, Papua New Guinea) and *A. auriculiformis* (Morehead River, Queensland and Coen River, Queensland) known to be outstanding in provenance trials at Ba Vi were used as controls. Cuttings were propagated from unselected seedlings of these superior provenances. Seedling controls, raised from seed collected from superior trees in *A. mangium* and *A. auriculiformis* plantations at Ba Vi, were also used as controls. Three trials were planted. The first trial tested three entries: cuttings derived from *A. auriculiformis* seedlings (Morehead River, Queensland provenance), cuttings derived from seedlings of *A. mangium* (Pongaki, Papua New Guinea provenance), and cuttings from 30 hybrid clones. This trial used a randomised complete block design with three replicates of 30-tree (5 × 6) plots.

The second trial tested five entries: a mixture of hybrid clones, seedling cuttings of *A. mangium* (Pongaki provenance), and seedling cuttings of *A. auriculiformis* (Coen River, Queensland provenance) as well as seedlings of local seed sources of *A. mangium* and *A. auriculiformis*. This trial used four replicates with each entry represented by a line plot of ten trees in each replicate.

The third trial tested 21 entries: 19 selected hybrid clones, seedling cuttings of *A. mangium* (Pongaki provenance), and seedling cuttings of *A. auriculiformis* (Coen River, Queensland provenance) in a randomised complete block design with three replicates, each entry being represented by a line plot of ten trees in each replicate.

All three clonal trials used a spacing of 3 m between rows and 2 m between trees within rows.

Tree height (H) and diameter (D) at breast height (DBH) were measured by traditional methods. Cylinder volume (V) of stem was calculated by the formula

$$V = \frac{\pi D^2}{4} H$$

Stem straightness, branch size (smallest best), crown development (conical best), crown thickness (thickest best) and phyllode colour (yellowish worst, green best) were classified into 5 classes ranging from 0 (lowest quality) to 5 (highest quality).

The software programs Datachain and Genstat were used for data entry and analysis. Following the procedures of Williams and Matheson (1994), clonal heritability was calculated for the line-plot trial of individual clones as the ratio

$$\frac{\sigma^2_{\text{clones}}}{\sigma^2_{\text{phenotypes}}}$$

where σ^2_{clones} is the variance component for between-clone differences and $\sigma^2_{\text{phenotypes}}$ is total phenotypic variance (equal to the sum of σ^2_{clones} , the between-plots variance component and the within-plots variance component). The pure-species clonal entries were excluded from the data set for the calculation of clonal heritabilities.

Results and discussion

Growth and morphological characteristics

Crown form and bark colour of the hybrid trees were intermediate between those properties of the parent species. However, the hybrid trees tended to be close to *A. auriculiformis* in these characteristics. The phyllodes of *A. mangium* were large and wide (phyllode index calculated as length/width was 2.73–2.79) and those of *A. auriculiformis* longer and narrower (phyllode index 5.23–6.69), the phyllode index of the hybrid trees was intermediate at 3.09–3.37. The number of main veins on the phyllodes of *A. mangium* was 4 and that of *A. auriculiformis* 3, while the phyllodes of the hybrid trees had 3–4 main veins.

The most obvious character of the acacia hybrids was their rapid growth. Growth of hybrid trees in plantations at Song May (2.5 y old, mechanical site preparation) and Ba Vi (2.5 and 4.5 y old, manual site preparation) was significantly faster than that of the two pure species (Table 1). At the age of 4.5 y, the stem volume of the hybrid trees at Ba Vi was twice that of *A. mangium*.

Table 1. Growth of the hybrid trees of *Acacia mangium* and *A. auriculiformis* and the parent species in plantations at Ba Vi and Song May

Growth characteristic	Hybrid		<i>A. mangium</i>		<i>A. auriculiformis</i>	
	X*	CV (%)**	X	CV (%)	X	CV (%)
Ba Vi: manual site preparation						
2.5-y-old plantation						
- Tree height (m)	4.5	21.2	2.8	32.3		
- DBH (cm)	5.2	29.7	2.6	51.5		
- Stem volume (dm ³)	9.59		1.50			
4.5-y-old plantation						
- Tree height (m)	9.7	17.4	8.0	21.1		
- DBH (cm)	12.4	16.4	9.6	26.6		
- Stem volume (dm ³)	116.8		57.6			
Song May: mechanical site preparation						
3-y-old plantation						
- Tree height (m)	10.9	11.8	7.3	23.4	8.0	13.5
- DBH (cm)	9.3	20.4	5.6	34.7	6.2	6.5
- Stem volume (dm ³)	74.0		17.8		23.7	

* Mean value based on 30 individual trees.

**Coefficient of variation.

The coefficients of variation for height and diameter of the hybrid trees at the two sites were smaller than those of *A. mangium*. Thus, growth of the hybrid trees was more uniform than that of *A. mangium*, but somewhat less uniform than that of *A. auriculiformis* at Song May.

Paper pulp potential

Wood density and chemical composition of woods

Results presented in Table 2 show that the wood density of the acacia hybrid is intermediate between the values of the parent species but close to that of *A. auriculiformis*. Studies elsewhere have determined the wood density of *A. auriculiformis* to be 0.50–0.51 g cm⁻³, and that of *A. mangium* 0.42 g cm⁻³ (Logan 1981, Clark *et al.* 1994).

Research results of the Institute for Cellulose and Paper Industry of Vietnam during the period of 1976 to 1991 showed that percentage of cellulose in woods of *Styrax tonkinensis*, *Manglieta glauca*, *Eucalyptus exserta*, *E. camaldulensis* and *E. urophylla*, species used for pulp production in North Vietnam (Table 3), is lower than that of the acacia hybrid and *A. mangium* (Table 2). Moreover, with the a lower lignin content than three of the five species in Table 3 and a higher pentosans content than all of them, the hybrid shows great pulping potential.

Table 2. Wood density and chemical composition of *A. mangium*, *A. auriculiformis* and their hybrid trees

Character	<i>A. mangium</i>	<i>A. auriculiformis</i>	Hybrid
Wood density (g cm ⁻³)	0.414	0.469	0.455
Percentage of substances (% of dry wood weight):			
Cellulose (Kuschner)	49.05	47.64	49.00
Lignin (Klason)	22.55	25.65	25.65
Pentosans	22.27	20.60	20.52
Benzene solubles	3.50	4.54	3.35
1% NaOH solubles	12.67	13.11	13.50
Hot water solubles	3.43	3.58	3.65
Cold water solubles	2.42	1.88	2.40

Table 3. Density and chemical composition of species used as material for pulp and paper production in northern provinces of Vietnam

Species	Density (g cm ⁻³)	Cellulose (%)	Lignin (%)	Pentosans (%)	Ash (%)
<i>Styrax tonkinensis</i> *	0.37–0.41	43.36	23.40	14.76	0.61
<i>Manglietia glauca</i> *	0.38	46.56	30.17	17.16	0.80
<i>Eucalyptus exserta</i> *	0.60	41.01	31.79	17.80	0.34
<i>E. urophylla</i> (7 years old)**	0.57	47.64	25.46	19.21	0.62
<i>E. camaldulensis</i> (7 years old)**	0.55	48.10	27.50	19.60	0.66

* Data from 1976 (cited in Paper and Cellulose Technology Review No. 1. 1996).

**Data from 1991 (cited in Scientific Report of the Institute for Cellulose and Paper Industry and Pulp and Paper Company at Bai Bang, 12/1991).

Pulp yield and paper properties

Data presented in Table 4 show that pulp yield of the acacia hybrid was significantly higher than those of *A. auriculiformis* and *A. mangium* as well as those of 7-y-old *E. camaldulensis* and *E. urophylla* (which gave yields of 47.9 and 46.9% respectively).

Compared with *A. auriculiformis* and *A. mangium*, the hybrid trees are significantly better in all important paper properties such as breaking strength, folding endurance and brightness (Table 4). The results of this study confirm those of Yamada *et al.* (1992) in most of the parameters studied in the pulping and papermaking investigation although the ages of the hybrids were different.

Table 4. Pulp yield and properties of paper made from 4-y-old *A. mangium*, *A. auriculiformis* and their F₁ hybrid grown in plantations at Ba Vi

Property	<i>A. mangium</i>	<i>A. auriculiformis</i>	Hybrid
Screen yield (%)	47.14	47.14	51.10
Kappa number	20.6	21.0	21.0
Brightness after bleaching (%)	81	82	85
Breaking length (km)			
Unbleached	6.85	6.67	8.40
Bleached	6.54	5.66	7.10
Folding endurance			
Unbleached	440	820	1300
Bleached	350	417	790
Ash (%)			
Unbleached	1.3	1.5	1.2
Bleached	0.9	0.9	1.0

Clonal tests

The rooting ability of the 30 superior hybrid trees selected for clonal propagation varied widely. The lowest rooting percentage was only 3.4% and the highest 84.7%.

Data collected at the age of 30–32 months from trials in which rooted cuttings of bulk clones were planted either in blocks or in rows showed that height, diameter at breast height, and volume growth of hybrid clones in both trials were significantly higher than those of trees that originated either from rooted cuttings or seedlings of the two pure species (Table 5).

Table 5. Growth of mixtures of hybrid acacia clones in comparison with pure species at Ba Vi

Species	Tree height		DBH		Stem volume (dm ³)
	H (m)	CV (%)	D (cm)	CV (%)	
Plots of 30 trees (30 months)					
Hybrid cuttings (mix of ramets from 30 selected hybrid clones)	8.70	7.9	8.74	11.3	52.2
<i>A. mangium</i> cuttings (Pongaki, PNG provenance)	6.89	10.1	7.39	16.0	29.6
<i>A. auriculiformis</i> cuttings (Morehead, Qld provenance)	5.34	15.1	5.66	16.2	13.4
Rows of 10 trees (32 months)					
Hybrid cuttings (mix of ramets from 19 selected hybrid clones)	9.35	7.8	9.10	9.3	66.8
<i>A. mangium</i> cuttings (Pongaki, PNG provenance)	7.51	9.0	7.51	18.2	44.3
<i>A. mangium</i> seedlings (local, Ba Vi)	7.49	8.5	8.31	13.6	48.9
<i>A. auriculiformis</i> cuttings (Coen, Qld)	6.59	11.1	6.41	17.9	33.2
<i>A. auriculiformis</i> seedlings (local, Ba Vi)	6.18	7.3	5.34	14.4	25.9

The clonal test where rooted cuttings of selected hybrid clones were planted in ten-tree rows showed that growth and morphological characters such as stem straightness, branch size, and apical dominance varied greatly between clones and were highly heritable. Clonal heritabilities calculated for the 19 hybrid clones in this test were 0.82 for height and 0.64 for diameter.

Five clones in this trial grew significantly faster than the remaining 14. The volume growth of the best clone surpassed the average volume of all trialed clones by 25%, and that of the worst clone by 53%. The best clone also had the highest quality scores for the other morphological characters assessed. Facilities for mass propagation of cuttings of the best hybrid clones have now been set up at several locations, and since January 1995 over 100 ha of trials and pilot plantations of these clones have been established on a range of site types in Northern, Central and Southern Vietnam.

Variability of F_1 hybrids compared with parent species and F_2 hybrids

A distinct advantage of F_1 hybrid clones is that trees within a clone are more uniform than trees of pure species that originated from either cuttings or seedlings. Notably, variation between trees originating from seeds of the F_2 generation (open-pollinated seed collected from F_1 hybrid individuals, which probably include F_2 hybrids and back-crosses to both species) is much higher than that between trees originating from seeds of the parent species (Table 6). In terms of morphological characters, segregation in the F_2 generation is also obvious. Therefore, seedlings of the F_2 generation are not suitable for use as planting material for wood-producing plantations.

Table 6. Coefficients of variation (CV%) of F_1 hybrid seedlings, F_1 hybrid cuttings, parent species seedlings and cuttings and F_2 seedlings at Ba Vi

Type of material	DBH CV (%)	Height CV (%)
Cutting-derived F_1 hybrids		
Mixture of 30 clones, clone trial 1	11.3	7.9
Mean value of 19 separate clones in clone trial 3, at 10 months	16.3	10.3
Mean value of 19 separate clones in clone trial 3, at 30 months	8.6	4.7
Cutting-derived pure species (32 months)		
- <i>A. mangium</i>	18.2	9.0
- <i>A. auriculiformis</i>	17.9	11.1
Seedling-derived pure species (32 months)		
- <i>A. mangium</i>	13.6	8.5
- <i>A. auriculiformis</i>	14.4	7.2
Seedling-derived F_2 hybrids (10 months)	32.3	28.1

Propagation by tissue culture of meristems

Axillary shoots collected from the best five tested clones were successfully vegetatively propagated by tissue culture (Nguyen Ngoc Tan *et al.* 1997). Development of bipinnate leaves in tissue culture suggested that significant rejuvenation had taken place. $1/2$ MS (Murashige and Skoog medium) + 0.1 mg/l IBA (indole-3-butyric acid) and $1/2$ MS + 1 mg/l ABT (a plant growth regulator, name not known to the author), were both suitable rooting media for hybrid plantlets. Rooting of individual plantlets was also stimulated by dipping in solutions of IBA or ABT at high concentration (500 mg l^{-1}). Using these methods, the rooting percentage of plantlets after 12 days reached values of 75%.

Conclusion

Wood density and morphological characters of natural hybrids of *A. mangium* $\times$ *A. auriculiformis* are intermediate between the values of the two pure species and a significant heterosis effect in the growth of natural hybrids was evident.

For paper produced by the sulphate method, paper pulp yield, breaking strength, folding endurance and brightness were higher for the hybrid than for the two parent species and alternative pulpwood species such as *Styrax tonkinensis* and *Manglietia glauca*.

The rooting percentage of hybrid cuttings varied greatly among different clones. Growth of hybrid cuttings was significantly higher than that of seedlings and cuttings of the two parent species. The coefficient of variation in growth of F_1 hybrid cuttings was lower than those of seedlings and cuttings of the parent species; and that of F_2 generation hybrid seedlings was much higher, making the latter unsuitable as a seed source for plantation forestry.

Substantial differences were observed in growth and morphological characters among F_1 hybrid clones. Selection of superior hybrid trees integrated with clonal tests identified some fast-growing clones which were uniform in terms of their morphological characteristics. These clones show great promise for clonal forestry in Vietnam and are now under test in a range of environments.

Mass propagation and rejuvenation of hybrid clones can be carried out using MS media with IBA or ABT.

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