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Full Length Research Paper

Radial variation of anatomical properties in 8-year-old clones of Acacia hybrid (*A. mangium* x *A. auriculaeformis*)

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Abstract

The radial variation of anatomical properties in three clones (HD3, K47 and H4) of 8-year-old Acacia hybrid (*Acacia mangium* x *Acacia auriculaeformis)*, grown by the Mysore Paper Mills, Bhadravati, Karnataka (India), is reported. The fibre and vessel morphology were studied from pith to periphery and variations between the clones were analyzed statistically. Various anatomical properties were also compared with the pure forms of *A. mangium* as well as *A. auriculaeformis* of identical age. Certain anatomical parameters such as fibre length, fibre diameter, fibre lumen diameter and vessel diameter showed significant radial variation from pith outwards. However, vessel frequency did not exhibit much radial variation. Fibre length was found to be positively correlated with vessel diameter, vessel element length and negatively correlated with fibre diameter and fibre lumen diameter. The correlation coefficients between various anatomical parameters were also computed and analyzed statistically. The results showed that inter clonal variation in various anatomical properties was significant except fibre lumen diameter and vessel diameter. All the clones showed longer fibres (1001-1078 µm) compared to pure forms of *A. mangium* (995 µm) and *A. auriculaeformis* (828 µm). Runkel ratio (0.644-0.679) and shape factor (0.443-0.471) values obtained in the present study are within the prescribed range to produce pulp of reasonable quality.

Key words: Acacia hybrid, *A. mangium*, *A. auriculaeformis*, clones, fibre and vessel morphology, Runkel ratio.

INTRODUCTION

Acacia auriculaeformis Cunn. Ex Benth was introduced in India in 1946 and now has naturalized in this part of sub-continent (Rai, 1995). It is an evergreen and well adapted to wide range of rainfall and soil conditions. It is a very useful species for reforesting the degraded nutrient deficient soils. Another Acacia species (*A. mangium* Willd.) was introduced in Indian subcontinent during 1984-85 and found to be well adapted with better form and growth rate and adaptable to wide geo-climatic conditions (Damodaran and Chacko, 1996). This generated wide interest for extensive plantation programmes in the states of Karnataka (Rai, 1995) and Kerala (Damodaran and Chacko, 1996; Patil et al. 2012) in India. Due to its excellent vigor and adaptability, *A. mangium* has also been one of the favoured reforestation species in Sabah, Malaysia (Rufelds, 1987). This species needs good site with

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deep fertile soil for better growth.

A. mangium x *A. auriculaeformis* hybrid trees were first spotted at Ulu Kukut, Sabah in 1971. These trees possess some of outstanding intermediate characteristics of their parents such as better stem form and longer clear bole height than *A. auriculaeformis* and lighter branching, circular trunks, smoother bark with white colour and smaller phyllodes compared with *A. mangium* (Rufelds, 1987). Denison and Kietzka (1992) reported that A. hybrids play an increasingly important role on the marginal sites needed for forestry. Kha (2000) reported that in Vietnam, the stem volume of A. hybrids is 2-3 times greater than that of *A. mangium* and 3-4 times greater than that of *A. auriculaeformis* of the same age.

The Mysore Paper Mills Ltd. (MPM) is a state owned pulp and paper manufacturing unit located at Bhadravati in Shimoga district of Karnataka, India. The MPM has developed several clones of Acacia and its hybrids and grown them under large-scale plantations on degraded forest, C and D class of lands to meet its pulpwood requirement. During Acacia species trials, a pilot plot of *A. mangium* was established from the seeds of *A. mangium* where *A. auriculaeformis* was planted next to it. A few progenies showed distinct morphological characters and were quite conspicuous in their growth rate. The review of literature has indicated the evolvement of natural hybrids from these species, where these species was grown in close proximity (Mohamed Amanulla et al., 2004). Later, in many of *A. mangium* plots, natural hybrids of Acacia species were recorded. These A. hybrids originated from *A. mangium* as mother were designated as 'Mangi-auriculis' (Mohamed Amanulla et al., 2004).

The anatomical, physical and mechanical properties of plantation grown pure forms of *A. mangium* and *A. auriculaeformis* have been studied and reported by many researchers (Kazmi et al., 1990; Kazuko et al. 2012; Kumar et al., 2006; Midon et al., 2002; Mohd. Hamami Sahri et al., 1993; Shukla et al., 2007a, 2007b; Varghese et al., 1999). A few studies have also been reported on various properties of Acacia hybrid (Ismail and Farawahida, 2007; Rusli et al. 2013). Recently, Sharma et al. (2015) have studied the physical and mechanical properties of three Acacia hybrid clones of 8-year-old. However, limited information is available on the radial variation of anatomical properties of Acacia hybrid (Hemavathi et al., 2006; Rao and Sujatha, 2004; Rao et al., 2007; Shashikala and Rao 2007; Yahya et al., 2010) and very meager information available on Acacia hybrid clones (Kha et al. 2012; Kim et al. 2011). In view of above, there was a need to study the anatomical properties for better selection and improvement of these hybrid clones for promoting specific end uses. The present studies were undertaken to understand the pith to periphery (radial) variations in anatomical features of three clones of Acacia hybrid *(A. mangium* x *A. auriculaeformis)*.

MATERIALS AND METHODS

Five trees each were selected from three clones (HD3, K47 and H4) of *Acacia* hybrid (*A. mangium* x *A. auriculaeformis)*, developed by the Mysore Paper Mills Ltd. (MPM) located in Bhadravati, Karnataka, India (Anon., 1990; Mohamed Amanulla et al., 2004)**.**These clones were grown at three nearby locations (HD3 at Heddur, K47 at Kanive and H4 at Halawani in Karnataka state of India) having 2000-3000 mm annual rainfall and situated at latitude (N) 13º38'46" and longitude (E) 75º17'35". These clones were grown in red, loam and lateritic soil, deep and with well drainage.

The radial variation of anatomical properties were studied using small billets from each tree. From each of the billet, 5 cm thick discs were cut and 2 cm wide radial strips passing through the pith were prepared. From each radial strips, three small wood blocks that is inner position (near pith), middle position and outer position (near bark) were prepared. Slivers were made for maceration from different radial positions for studying the fibre and vessel morphology (Jane 1970). Fibre length, fibre diameter, fibre lumen diameter, vessel diameter and vessel element length were measured using Image Analysis System (Lieca Model No. Q500) in the interactive measurement mode. About thirty measurements were taken for each anatomical characteristic from each position. Fibre wall thickness was computed by deducting fibre lumen diameter from fibre diameter. Vessel frequency was determined by counting the number of vessels per mm^2 per field of view using 2.5× lens. An average value was calculated from ten fields of view per position. Certain paper quality parameters such as Runkel ratio and shape factor were computed using following formula:

$$\text{Runkel ratio} = \frac{2w}{ld} \quad (1)$$

(2)

where, w is wall thickness, ld is fibre lumen diameter and fd is fibre diameter. The statistical analysis of data was carried out using Sigma Stat (Ver. 3.5, Systat Software Inc., 2006) software. The data were analysed using single factor ANOVA for finding significance of the character with reference to radial variations distances and correlation coefficients were also determined between the characters to understand their inter dependence.

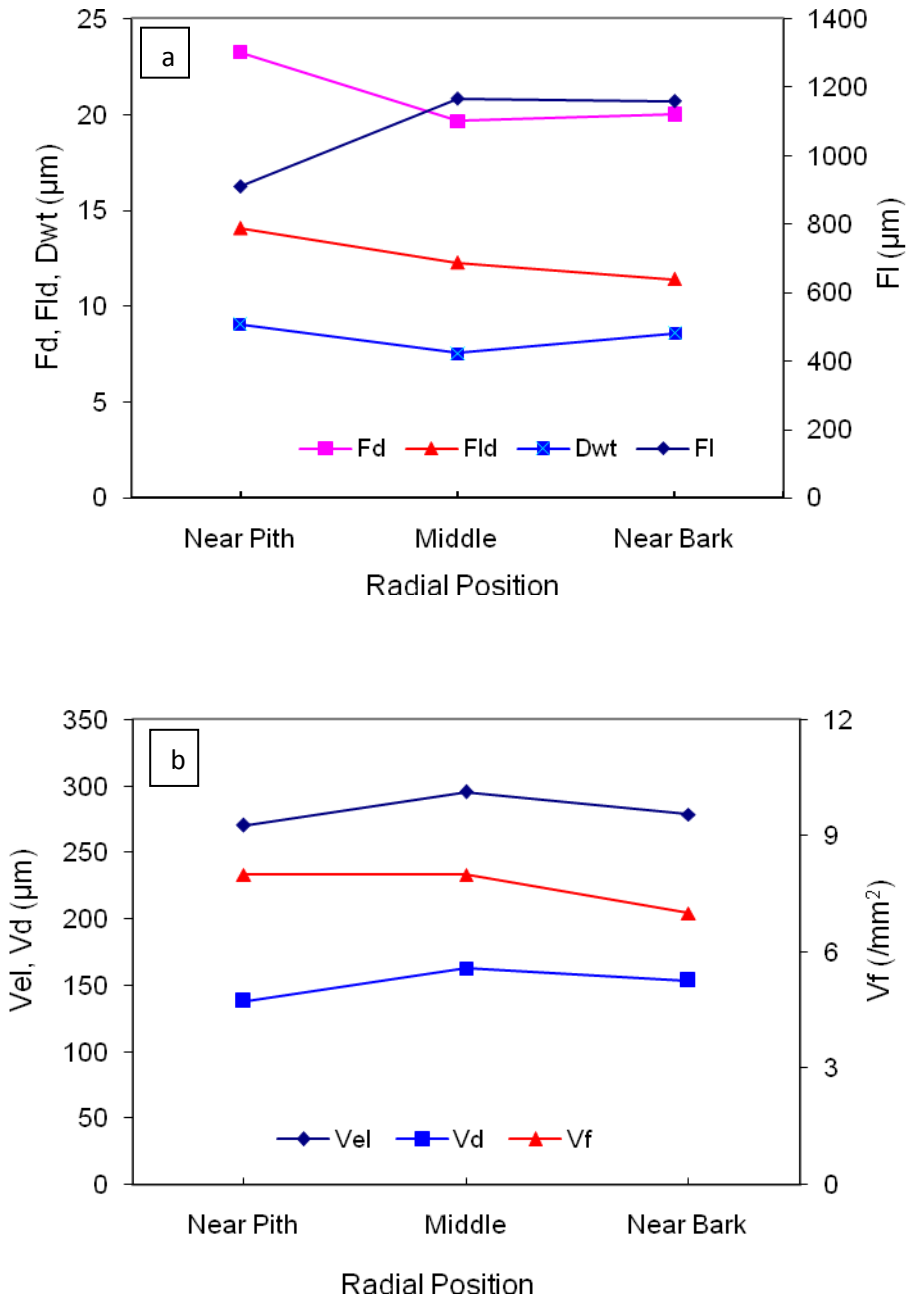

Figure 1. Radial variation in fibre morphology (a) and vessel morphology (b) in HD3 clone. Fl = Fibre length, Fd = Fibre diameter, Fld = Fibre lumen diameter, Vf = Vessel frequency, Vel = Vessel element length, Vd = Vessel diameter.

RESULTS AND DISCUSSION

Gross structure

The wood was found to be diffuse-porous. Growth rings were distinct and delimited by a thin line of parenchyma cells. Vessels were small to moderately small and numbered in the range of 4-13/mm^2, more or less evenly distributed, in some rings crowding of the vessels was also observed. Vessels were solitary and in radial multiples of 2-3 and diagonally arranged, round to oval in outline. Coloured deposits were present. Vessel lines were

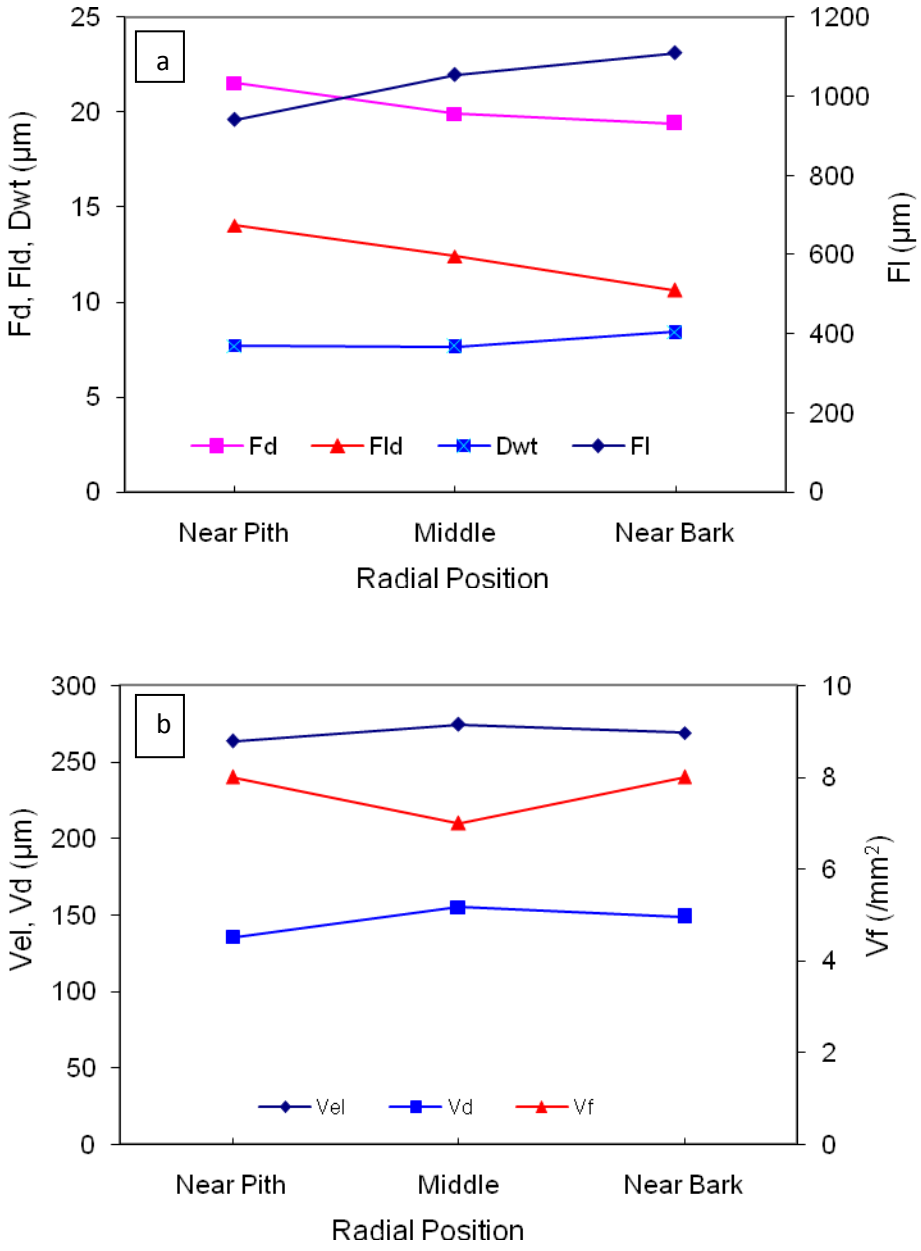

Figure 2. Radial variation in fibre morphology (a) and vessel morphology (b) in K47 clone. Fl = Fibre length, Fd = Fibre diameter, Fld = Fibre lumen diameter, Vf = Vessel frequency, Vel = Vessel element length, Vd = Vessel diameter.

seen distinct on the longitudinal surface. Parenchyma/soft tissues were not visible to the eye but distinct under the hand lens as scanty thin sheaths round the vessels and also aliform. Rays were fine, distinct under the hand lens, widely spaced and lighter in colour against the background, found to be uniformly distributed.

Anatomical descriptions

Figures 1-3 depict the radial variation in fibre and vessel morphology for HD3, K47 and H4 clones respectively. In HD3 clone (Figure 1a), fibre length showed increasing trend from pith to middle portion of the disc and subsequ-

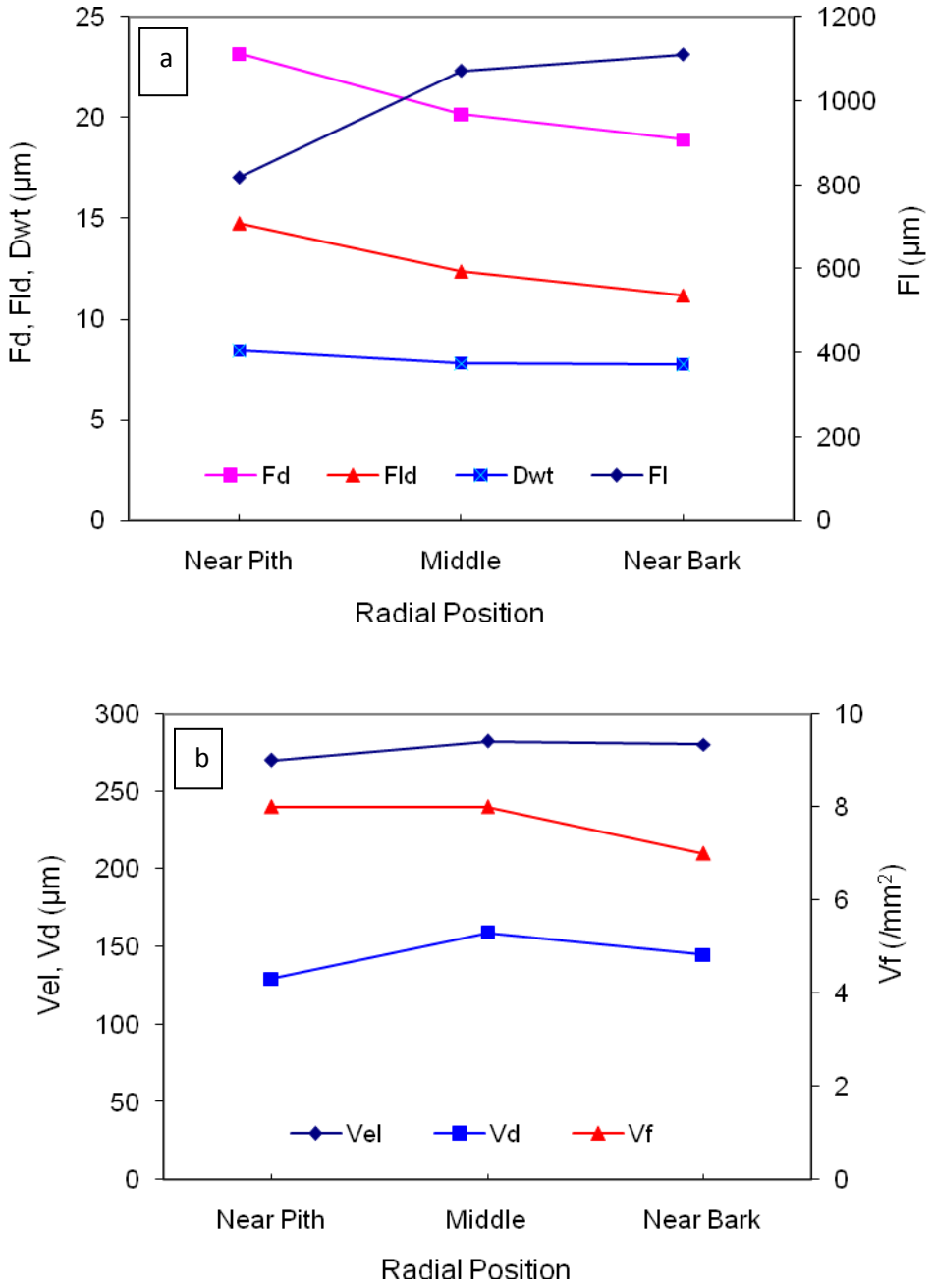

Figure 3. Radial variation in fibre morphology (a) and vessel morphology (b) in H4 clone. Fl = Fibre length, Fd = Fibre diameter, Fld = Fibre lumen diameter, Vf = Vessel frequency, Vel= Vessel element length, Vd = Vessel diameter.

ently not much change to outer portion. Fibre diameter showed decreasing trend in middle position and slight increasing trend towards bark. Fibre lumen diameter showed decreasing trend from pith outwards. Double wall thickness showed decreasing trend from pith to middle and then increased slightly. From Figure 1b, it is clear that not much variation was observed in vessel frequency. Vessel diameter showed increasing trend in the middle position and slight decreasing trend towards outer portion. Vessel element length showed increased and decreased trend.

In K47 clone (Figure 2a), the increasing trend of fibre length whereas fibre diameter and fibre lumen diameter showed slight increase and decreasing trend from pith

Table 1. Comparative radial variation in average values of anatomical properties of Acacia hybrid clones.

Clone No.	Properties	Position from pith			Significance at test
		Near pith	Middle	Near bark	
HD3	Fibre length (µm)	910 (7.05)	1166 (7.18)	1159 (8.15)	***
	Fibre diameter (µm)	23.24 (0.28)	19.67 (0.28)	20.04 (0.24)	***
	Fibre lumen diameter (µm)	14.07 (0.25)	12.26 (0.26)	11.39 (0.22)	***
	Double wall thickness (µm)	9.04 (0.19)	7.53 (0.13)	8.591 (0.18)	***
	Vessel frequency(/mm^2)	8 (0.15)	8 (0.16)	7 (0.20)	*
	Vessel diameter (µm)	138 (2.00)	163 (2.67)	153 (3.38)	***
	Vessel element length (µm)	270 (4.20)	296 (4.30)	278 (5.01)	***
K47	Fibre length (µm)	941 (9.79)	1054 (9.78)	1110 (7.41)	***
	Fibre diameter (µm)	21.52 (0.33)	19.91 (0.39)	19.41 (0.26)	***
	Fibre lumen diameter (µm)	14.03 (0.26)	12.41 (0.22)	10.63 (0.20)	***
	Double wall thickness (µm)	7.70 (0.17)	7.66 (0.31)	8.43 (0.17)	NS
	Vessel frequency(/mm^2)	8 (0.26)	7 (0.23)	8 (0.19)	NS
	Vessel diameter (µm)	136 (2.12)	155 (2.35)	149 (2.83)	***
	Vessel element length (µm)	264 (5.17)	275 (4.07)	269 (4.13)	NS
H4	Fibre length (µm)	819 (7.39)	1073 (6.94)	1111 (6.81)	***
	Fibre diameter (µm)	23.17 (0.39)	20.17 (0.31)	18.94 (0.24)	***
	Fibre lumen diameter (µm)	14.74 (0.37)	12.38 (0.31)	11.20 (0.26)	***
	Double wall thickness (µm)	8.43 (0.17)	7.79 (0.13)	7.74 (0.12)	***
	Vessel frequency(/mm^2)	8 (0.27)	8 (0.23)	8 (0.23)	*
	Vessel diameter (µm)	130 (2.06)	159 (2.44)	145 (2.91)	***
	Vessel element length (µm)	270 (4.54)	282 (4.73)	280 (4.11)	NS

Note: Values in parenthesis indicate standard error; NS = Not significant. * = significant ($p<0.05$), ** = significant ($p<0.01$) and *** = significant ($p<0.001$).

outwards. Double wall thickness was almost constant from pith to periphery. Figure 2b shows that vessel frequency decreases in the middle position and increases towards bark. Vessel element length and vessel frequency showed no change from pith to periphery.

H4 clone (Figure 3a) showed significant radial variations in fibre length, fibre diameter and fibre lumen diameter. Fibre lumen diameter showed decreasing trend from pith outwards. Fibre length and fibre diameter showed decreasing trend whereas double wall thickness showed decreasing trend from pith outwards. It is seen from Figure 3b that vessel element length showed increasing trend from pith to middle and decreasing trend outwards whereas vessel frequency showed no significant change**.**

Table 1 lists the average values along with their statistical significance of different anatomical properties for all the three clones. It is observed from this table that in HD3 clone, all the anatomical parameters showed significant radial variation from pith to periphery. In clone K47, except double wall thickness, vessel frequency and vessel element length, while all other parameters showed significant radial variations. Similarly, in H4 clone, all anatomical parameters related to fibre and vessel showed significant variations except vessel element length.

The comparison of average values of different fibre and vessel parameters of all the three clones are shown in Table 2. Most of the anatomical parameters were having higher values for HD3 clones. No statistically significant difference was observed between the three clones with respect to fibre lumen diameter and vessel frequency. However, other parameters such as fibre length, fibre diameter, double wall thickness, vessel diameter and vessel element length showed significant difference among the clones.

Table 3 depicts a comparison of Acacia hybrid clones along with hybrid and pure forms of *A. mangium* and *A. auriculaeformis*. It is observed that Acacia hybrid fibres were 4.3 and 25.4% longer than pure forms of *A. mangium* and *A. auriculaeformis* respectively (Rao and Sujatha, 2004; Rao et al., 2007) but slightly lower than the Acaia hybrid (Hemavathi et al., 2006). Similar observations was made by Yahya et al. (2010) in an earlier study in Indonesia in which fibres of Acacia hybrid clones were significantly longer than those of both parents.

The data on radial variation of all the three clones were

Table 2. Average values of different anatomical parameters of Acacia hybrid clones.

Properties	Clone Number			Significance
	HD3	K47	H4	
Fibre length (µm)	1078 (7.07)	1035 (6.19)	1001 (7.35)	***
Fibre diameter (µm)	20.98 (0.17)	20.28 (0.19)	20.76 (0.20)	*
Fibre lumen diameter (µm)	12.58 0.15)	12.36 (0.15)	12.77 (0.19)	NS
Double wall thickness (µm)	8.39 (0.10)	7.93 (0.13)	7.99 (0.08)	**
Vessel frequency (/mm^2)	7 (0.10)	8 (0.13)	7 (0.14)	NS
Vessel diameter (µm)	151 (1.65)	146 (1.46)	144 (1.55)	**
Vessel element length (µm)	281 (2.65)	269 (2.59)	277 (2.59)	**

Note: Values in parenthesis indicate standard error; NS = Not significant, * = significant ($p<0.05$), ** = significant ($p<0.01$) and *** = significant ($p<0.001$).

Table 3. Comparison of anatomical properties of pure forms of *A. mangium, A. auriculaeformis, A. mangium* × *A. auriculaeformis* hybrid and their clones.

Properties	*A. mangium* x *A. auriculaeformis* hybrid clones (present study)			*A. mangium* x *A. auriculaeformis* hybrid[1]	*A. mangium*[2]	*A. auriculaeformis*[3]
	HD3	K47	H4			
Fl (µm)	1078	1035	1001	1107	995	828
Fd (µm)	20.98	20.28	20.76	19.85	20.87	17.4
Fld (µm)	12.58	12.36	12.77	13.9	13.97	10.5
Dwt (µm)	8.39	7.93	7.99	5.95	6.90	6.9
Vf (/mm^2)	7	8	7	5	9	11-12
Vd (µm)	151	146	144	174.5	129	131-135
Vel (µm)	281	269	277	327	328	252
Runkel ratio	0.679	0.661	0.644	0.428	0.494	0.573
Shape factor	0.471	0.443	0.456	0.342	0.381	0.424

Fl = Fibre length, Fd = Fibre diameter, Fld = Fibre lumen diameter, Dwt = Double wall thickness, Vf = Vessel frequency, Vd = Vessel diameter, Vel = Vessel element length.
[1]Hemavathiet al., 2006; [2] Rao and Sujatha, 2004; [3] Rao et al., 2007.

pooled and simple correlation was performed to find relationships among different anatomical parameters. Table 4 lists the correlation coefficients between different anatomical properties. It can be seen from the table that fibre length is negatively correlated with fibre diameter (r = - 0.287) and fibre lumen diameter (r = - 0.288), positively correlated with vessel diameter (r = 0.254) and vessel element length (r = 0.142). Fibre diameter is positively correlated with fibre lumen diameter (r = 0.782) and double wall thickness (r = 0.467). Fibre lumen diameter is found to be negatively correlated with double wall thickness (r = - 0.134). Vessel diameter is positively correlated with vessel element length (r = 0.143).

Runkel ratio and shape factor

Runkel ratio and shape factor are two important anatomical parameters which help in predicting the expe-

Table 4. Correlation coefficients between different anatomical properties. Corresponding p-values are given in the parenthesis.

Property	Fd	Fld	Dwt	Vf	Vd	Vel
Fl	-0.287 (0.000)	-0.288 (0.000)	-0.072 (0.008)	-0.035 (0.467)	0.254 (0.000)	0.142 (0.000)
Fd		0.782 (0.000)	0.467 (0.000)	0.067 (0.165)	-0.065 (0.017)	0.006 (0.818)
Fld			-0.134 (0.000)	0.062 (0.195)	-0.080 (0.003)	0.046 (0.089)
Dwt				0.010 (0.830)	0.001 (0.962)	0.056 (0.041)
Vf					0.031 (0.516)	0.020 (0.674)
Vd						0.143 (0.000)

Fl = Fibre length; Fd = Fibre diameter; Fld = Fibre lumen diameter; Dwt = Double wall thickness; Vf = Vessel frequency; Vd = Vessel diameter; Vel = Vessel element length.

cted quality of pulp and paper. Average value of Runkel ratio for HD3 clone was 0.679 with a total range from 0.513 to 0.774 and is highest among three clones. In K47 clone, the overall average value of 0.661 was observed. Clone H4 showed an average value of 0.644.The shape factor ranged from 0.391 to 0.511 with an overall average value of 0.471 in HD3 clone. In clone K47, lowest value of 0.443 and highest value of 0.465 with an overall average value of 0.458 was observed. In clone H4, the Runkel ration was observed to be varied from 0.402 to 0.510 with an average value of 0.456. Among the three clones, HD3 showed highest value of 0.471 and H4 clone showed lowest value of the shape factor (0.456). The analysis showed that the Acacia hybrids having lower values of Runkel ratio and shape factor are good fibre resource for better strength paper. Runkel ratio values obtained in the present study are within the prescribed range of 0.25 to 1.50 to produce pulp of reasonable quality (Singh et al., 1991).

CONCLUSIONS

The pith to periphery variations in fibre length, fibre diameter, fibre lumen diameter, double wall thickness, vessel frequency, vessel diameter and vessel element length were studied in three 8-year-old Acacia hybrid (*A. mangium* x *A. auriculaeformis*) clones. Fibre length, fibre diameter, fibre lumen diameter and vessel diameter showed significant variation from pith outwards whereas vessel frequency showed not much variation. The fibre length was found to be negatively correlated with fibre diameter and fibre lumen diameter, positively correlated with vessel diameter and vessel element length. Fibre diameter was positively correlated with fibre lumen diameter and double wall thickness. Fibre lumen diameter was negatively correlated with double wall thickness while vessel diameter was positively correlated with vessel element length. Inter clonal variation in various anatomical properties was observed to be statistically significant except fibre lumen diameter and vessel diameter. All the three clones showed longer fibres compared to the pure forms of *A. mangium* and *A. auriculaeformis*. Runkel ratio values obtained in the present study were within the prescribed range to produce pulp of reasonable quality.

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