

Some Morphological Characteristics of *Eucalyptus camaldulensis* Dehn. Clone A5 and Clone D1 at the Clonal Plantation in Eastern Thailand

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ABSTRACT

The genetic improvement of *Eucalyptus* species in Thailand has been developed at clone or variety levels. *Eucalyptus camaldulensis* Dehn. Clone A5 and Clone D1 are two of the most planted varieties at Sa Kaeo province in the eastern region of Thailand. Thus, the selection of clones which emphasize economic traits is not sufficient anymore. Wood density and leaf functional traits should be emphasized also because these characteristics directly affect yields of plantation. The studied area was a six-year-old clone A5 and D1 plantation with spacing of 3 m. x 3 m. in the village of Sa Kaeo Province, in eastern Thailand.

The basic wood density (WD) of A5 and D1 was 0.7364 and 0.6345 g/cm³, respectively. Stem volume was 0.0512 and 0.0577 m³/tree and the stem dry mass was 28.10 and 26.50 kg/tree, respectively. Although the stem volume of A5 was less than D1, their WD had significant statistical differences ($p < 0.05$) because the WD of A5 was higher than D1. Furthermore, the A5 provided more stem dry mass than D1, equal to 30.95 and 28.40 ton per hectare, respectively. For the leaves slenderness, A5 and D1 were 1.18 and 1.19 respectively, which had insignificant difference ($p > 0.05$) and the specific leaf weight was 0.0135 and 0.0120 g/cm², respectively, which had a significant statistical difference ($p < 0.05$). These results indicated that the leaf thickness of A5 was more than D1. It was affected positively by photosynthesis. The results suggest that wood density of stem and thickness of leaves in this study could be used further to improve the genetic *Eucalyptus*.

Keywords: *Eucalyptus camaldulensis*; Clonal plantation; Morphology; Eastern Thailand

Introduction

Eucalyptus is a fast growing plant that Thailand has experimentally planted since 1950. In 1989, the government announced the termination of terrestrial forest concessions; therefore, the private sector was promoted to grow more plants for supporting the demand of wood in the country. *Eucalyptus camaldulensis* Dehn was a plant growing faster than other species [1] and could be cut at less than 7 years old. In addition, its girth increased more than 10 cm. per year [2]. The genetic improvement of *E. camaldulensis* had been developed through clones, such as clone A5 and clone D1 (A5 and D1, respectively) which were widely planted in the eastern region. Most owners of plantations bought Eucalyptus clones for clonal trials and were selected for promoting the clones. The selected clones emphasized economic traits, such as fast growth, good natural pruning, small to medium branch size, and insect and disease tolerance [1]. Regarding the wood density, it is implied that the wood strength and elasticity, as well as breaking strength, is directly related to yields or dry matters [3]. Alternatively, wood density could be inherited. In addition, wood density varied according to stem diameter and high levels. It is also varied with the wood accumulation rate, age, management and site quality [4-7].

However, leaf functional traits directly influence light interception and photosynthesis, which are important to the growth of trees [8]. For a leaf's photosynthesis capacity, it is related to the development of cells and organs. The development of cells refers to the growth of chloroplast. The cell enlargement will affect the increase in leaf thickness and vary according to basic genetics [9]. The specific leaf weight, which is the proportion of dry weight of the leaf and surface area of the leaf [10], was used for analyzing cell enlargements. The leaf thickness was also related to the number of cell layers, which generally is at least 6-9 layers, including

palisade cells that could be enlarged with the development of intercellular space in spongy parenchyma [9]. If the layer of mesophyll is larger, it will be positively affected by the photosynthesis because more chlorophyll can be accumulated [11-13]. Higher chlorophyll per area unit creates a higher level of photosynthesis [14-16]. However, this does not include the thickness of the leaf from cutin or wax accumulation, which can be found in mature leaves but causes a reduction in transpiration of the leaf [17].

Part of the development of the leaf organ is lamina expansion, which is directly related to the leaf surface [9]. For the leaf surface analysis, the specific leaf area is used, which is the reverse value of the specific leaf weight [10] and the specific leaf area, used for displaying the number of area units that have photosynthesis in normal conditions [8]. It also implies that the leaf width affects the ability of light interception. The greater the width of the leaf, the greater the amount of light captured [18].

Moreover, the anatomy and physiological characteristics of the leaf are related to photosynthesis capacity. For instance, a leaf with a higher stoma volume and lower induced stomata and transpiration can be considered as the characteristic promoting gas exchange of the leaf [19-20]. This is including external factors too, such as the tree crown shapes, the crown of trees with large bushes and thickset, which all positively affect growth [21].

The study aimed to investigate the difference in volume and dry mass of the stems of A5 and D1 and the difference of stem wood density, leaf slenderness and thickness between both clones by studying a 6 year old *Eucalyptus camaldulensis* plantation with spacing of 3 m. x 3 m.

Materials and Methods

Description of A5 and D1

Both clones have the same type of stem and leaf, being cylindrical and having a straight stem, thin bark, soft white stem,

lanceolated leaf, acute leaf apex and an obtuse leaf base. For the distribution of leaves and branches, the A5 leaves have a clumped distribution along the branches while the D1 leaves have a regular distribution along the branches [22].

The studied areas

The study was conducted in the 3 m. x 3 m. of A5 and D1 plantations of Chong Kam (13°52'20" N and 102°16'104"E) in Sa Kaeo Province, eastern Thailand. The soil texture was sandy clay loam and coarsely sandy loam soil, respectively. The soil pH was approximately 5. The average annual rainfall was 1,468.8 millimeters; the average of rainy day was 129.5 days; the highest and lowest average temperatures were 39.2 and 12.7 °C. The dry period of 5 months was from November until March.

The sample plots

The sample plots were representative area of plantations and quite distant from the edge of the plantations. The size of the sample plot in both clones plantation was 45 m. x 45 m., consisting of 15 rows, each of which contained 15 positions of trees. Diameter at breast height (DBH) and total height of each tree within the sample plots were measured.

Sample tree selection and management

Sample tree selection

Sample trees were selected outside the plot for the further analyses. Number and size of sample trees in each plot were determined based upon the range of the existing DBH. There were 13 and 8 sample trees with DBH ranging between 2.10-13.28 cm. and 1.75-14.23 cm. for clones D1 and A5, respectively.

Sample tree management

Each selected sample tree was managed as follows:

- felling at ground level.
- measuring total height, height at the first living branch, height at diameter equal to 2, 5 and 10 cm.

- measuring diameters at ground level (0.0 m) , 0.30 m and every 1.0 m interval along the height of tree.

- separating the tree, including branch and leaves, at each height of measuring diameter.

- separating and weighing composition of tree in each section into stem, branches and leaves. Each of them were also sampled and weighed.

- collecting a 2-cm thickness disc of stem at 1.30 m.

- collecting 20-30 mature leaves of each sample tree.

Laboratory operation

All the samples taken from field work were oven dried at 103 °C for 24 hours or until the dried weight became stable [25], except the samples of 20-30 mature leaves of each sample tree which were dried out at 80 °C for 24 hours. Then, their dried weight was recorded.

Data analysis

The following calculations and analysis were determined.

- Converting dry weight of each component of the sample tree, and summing up for each of them.

- Calculating stem volume of each log using Smalian's Formula (2011) [23].

- Determining the relationship between DBH and DBH²Ht with dried weight of each component and total volume using the allometry relation in the form of a power equation.

- Determining the relationship between DBH and the ratio of volume between the limited stem diameter (V2, V5 and V10 for volume of stem up to diameter equal to 2 5 and 10 cm) and the total (VT) by using Richard's function as follows:

$$V_x/V_T = A * (1 - \exp^{(-h * DBH)^{\frac{1}{1-p}}}) \quad (\text{Eq.1})$$

Where A, h and p are constants value and x are 2.00, 5.00 and 10.00 cm.

Reducing the error was possible from the results of the analysis with the regression equation. The error that is possible is that the

volume value at the limited diameter is higher than the total volumes. Therefore, the Richard's function has been used for constructing the equation to estimate the adjusted value [31].

- Afterwards, the volume of the stems and the dried weight of the stems, branches, and leaves of the total trees in the sample plots in A5 and D1, are converted to the total stem volume and the dried weight per unit of area.

- Determining commercial stem wood density of each collected disc by using the following equation

$$WD (g/cm^3) = \frac{\text{oven dried weigh (g)}}{\text{green volume (cm}^3\text{)}} \quad (\text{Eq.2})$$

A commercial study of stem wood density was used at breast height, or 1.30 above the ground, on a piece of felled trees, or 10% of the total tree height [26]. The piece of wood was soaked with water and its volume was measured by water replacement, which reported that 1 gram of water equaled 1 cm³ [27],

- Processing the collected mature leaves of each sample tree as follows:

(1) Determining areas of each leaf by using the Image J program [28].

(2) Selecting leaves of each sample tree based upon the differences of their area being no greater than 1 cm²

(3) Specific leaf weight (SLW) which was the index of leaves thickness measurement. Determining leaf thickness, so called specific leaf weight (SLW), by using the ratio between dried weight (cm²) and area of the leaf (gram)

(4) Dividing each leaf into two parts at the widest point of the leaf, the leaf apex and leaf base. The ratio between actual leaf surface and the rectangular shape produced from the widest and length of each part was calculated. The leaf slenderness equals the ratio between the previously mentioned ratio of base and apex.

Statistical analysis.

The difference of WD, the leaf slenderness and thickness between clone A5 and D1, was determined by using a Two sample- T test, which was performed in MINITAB version 14 [29]. A significance level of 0.05 was used for both clones comparison.

Results and Discussion

Equation for estimating and adjusting the stem volume

Table 1 shows the volume allometric equation in the power equation which used the DBH and DBH²H as an independent variable of A5 and D1, setting the correlation of determination (R²) between 0.9224-0.9985 and 0.9991-0.9992, respectively. It was found that the equation used the DBH and DBH²H as independent variables set quite similar to R², suggesting that they can be substitutes. The proportion of volume of the limited diameter for both clones, such as 2.00, 5.00 and 10.00 cm and total stem volume, had a relationship with DBH in the form of an s- shape curve (Fig. 1). Accordingly, the growth of trees was in the form of exponential equation [30]. Regarding the adjusted stem volume, the adjusted volume equation with a limited diameter (Table 2)

Table 1. The stem volume allometric equation in form of power equation of A5 and D1.

Clone	Allometric equation	R ²
A5	$y = 0.000197 * DBH^{2.483857}$	0.9924
	$y = 0.000067 * DBH^2 H^{0.930317}$	0.9985
D1	$y = 0.000198 * DBH^{2.432726}$	0.9992
	$y = 0.000073 * DBH^2 H^{0.913185}$	0.9991

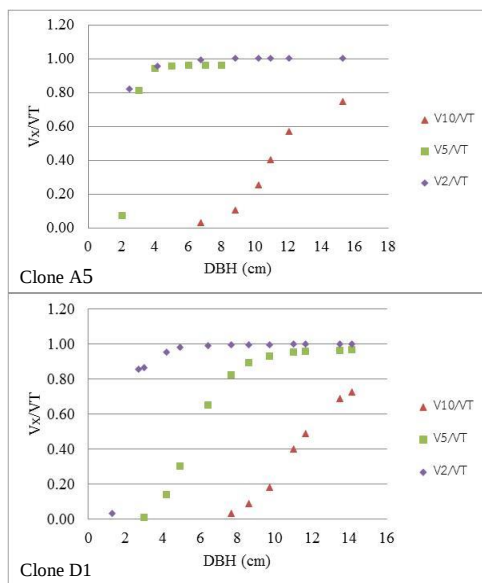


Fig. 1. The relationship line between the proportion of limited diameter volume and total volume and DBH of A5 and D1.

Equation for estimating the dry mass of stem, branch and foliage

The A5 and D1 clones had the biomass allometric equation in the power equation, which used the DBH and DBH^2H as an independent variable and provided the coefficient of determination (R^2) between 0.8935-0.9982 and 0.9219-0.9978, respectively (Table 3). The stem biomass equations of both clones showed that the equation which used height combined with DBH as a single variable provided higher R^2 than the equation which used only DBH as an independent variable. Because of the allometric equation which used both DBH and tree height as an independent variable had a close correlation with stem form [32]. Regarding the biomass allometric equation of the branch and leaf, the R^2 was less than the stem biomass allometric equation because the tissues of the branch and leaf were not permanently with the stem [33].

Table 2. Equation of the adjusted estimation in form of Richards function.

Equation of Clone A5	
$V_{10}/V_T = 0.792 (1 - \text{Exp}(-0.5697 \cdot DBH))^{1/(1-0.997)}$	
$V_5/V_T = 0.964 (1 - \text{Exp}(-1.0390 \cdot DBH))^{1/(1-0.995)}$	
$V_2/V_T = 0.999 (1 - \text{Exp}(-0.7980 \cdot DBH))^{1/(1-0.254)}$	
Equation of Clone D1	
$V_{10}/V_T = 0.883 (1 - \text{Exp}(-0.4645 \cdot DBH))^{1/(1-0.993)}$	
$V_5/V_T = 0.966 (1 - \text{Exp}(-0.7166 \cdot DBH))^{1/(1-0.974)}$	
$V_2/V_T = 0.990 (1 - \text{Exp}(-1.9956 \cdot DBH))^{1/(1-0.975)}$	

General characteristics of A5 and D1

From Table 4, the study found that the average of DBH was 9.19 and 10.14 centimeters. Furthermore, the average of H was 13.80 and 12.74 meters, respectively, including the H/D ratio, which was 1.50 and 1.26, and the average width of crown cover was 4.87 and 6.24 meters, respectively. A5 had a higher ratio than D1 and it also had less width of crown cover than D1. Additionally, A5 had more of the crown's ability to contribute to stem growth than D1 had, which crown cover led this characteristic of A5 to the least competitive trees [34].

Table 3. Biomass allometric equation in form of power equation for estimating dry weight of stem, branch and leaf of A5 and D1.

Part of tree	Allometric equation	R^2
Clone A5		
stem	$y = 0.103227 \cdot D^{2.505506}$	0.9925
	$y = 0.034918 \cdot D^2 H^{0.938251}$	0.9982
branch	$y = 0.030759 \cdot D^{2.062028}$	0.9222
	$y = 0.013857 \cdot D^2 H^{0.757891}$	0.8935
foliage	$y = 0.018481 \cdot D^{2.098805}$	0.9619
	$y = 0.007825 \cdot D^2 H^{0.778624}$	0.9495
Clone D1		
stem	$y = 0.117408 \cdot D^{2.326824}$	0.9963
	$y = 0.044770 \cdot D^2 H^{0.874092}$	0.9978
branch	$y = 0.033206 \cdot D^{1.969570}$	0.9448
	$y = 0.014893 \cdot D^2 H^{0.737505}$	0.9401
foliage	$y = 0.012721 \cdot D^{2.045729}$	0.9206
	$y = 0.005451 \cdot D^2 H^{0.768470}$	0.9219

Note: D is DBH

Table 4. Mean of DBH, H, and H/D ratio of A5 and D1.

Range of DBH (cm)	Mean of			H/D ratio
	DBH (cm)	H (m)	crown cover width (m)	
Clone A5				
2.10-13.28	9.19	13.79	4.87	1.54
Clone D1				
1.75-14.23	10.25	12.71	6.24	1.26

Yields in terms of the stem volume and dry mass

The A5 and D1 clones provided the stem volume of 0.0512 and 0.0577 m³/tree or 56.36 and 61.86 m³/ha, respectively. For the stem volume with a limited diameter (V₂, V₅, V₁₀) it was found that A5 provided greater stem volume at all limited diameters than D1 did (Table 5). For the dry mass of the stem branch and foliage or above ground biomass, it was found that A5 and D1 provided the stem DW of 28.10 and 26.50 kg/tree or 30.95 and 28.40 ton/ha, respectively (Table 6). These show that the stem volume of A5 was less than D1 but provided more stem DW than D1. In addition, A5 provided higher DW of branch and foliage than D1. The stem volume productions of both clones, with spacing 3 m x 3 m in Sa Kaeo province, were consistent with other concerned studies, such as the six-year old Eucalyptus plantation in Chachoengsao Province. By a thinning out of 75%, it provided the stem volume at the diameter with more than 5 cm between 55.94-62.50 m³/ha [35]. An *E. camaldulensis* plantation in Ratchburi Province, which was four years old with a spacing of 2 m x 4 m, provided the stem dry mass of 27.41 ton/ha [36]; an *E. Camaldulensis* plantation in Krabi Province, which was five years old with a spacing of 4 m x 4 m, provided the stem dry mass of 24.25 ton/ha [37]; and an *E. camaldulensis* plantation in Kalasin Province, with an age of 4.39 years and moderate soil fertility conditions, provided

the stem dry mass of 35.02 ton/ha [38]. Thus stem biomass is affected by stand density, age and site quality which are factors related to competition and carrying capacity [36, 38].

Table 5. Stem volume of 6-year old A5 and D1 with spacing of 3 m x 3 m.

Diameter (cm.)	Stem Volume		
	m ³ /tree	m ³ /rai	m ³ /ha
Clone A5			
≥ 10	0.0141	2.48	15.49
≥ 5	0.0481	8.47	52.93
≥ 2	0.0511	9.00	56.23
Total	0.0512	9.02	56.36
Clone D1			
≥ 10	0.0231	3.96	24.77
≥ 5	0.0541	9.27	57.96
≥ 2	0.0571	9.79	61.20
Total	0.0577	9.90	61.86

Table 6. Dry mass of stem, branch and foliage of 6 year old A5 and D1 with spacing of 3 m x 3 m.

Part of tree	Dry mass		
	kg/tree	ton/rai	ton/ha
Clone A5			
Stem	28.10	4.95	30.95
Branch	3.03	0.53	3.34
Foliage	1.99	0.35	2.19
Total	33.13	5.84	36.48
Clone D1			
Stem	26.50	4.54	28.40
Branch	3.21	0.55	3.45
Foliage	1.48	0.25	1.58
Total	31.20	5.35	33.43

The basic wood density of stem

Table 7 shows that A5 and D1 clones had an average WD of 0.7364 and 0.6345 g/cm³, respectively, and the average WD between the two clones was statistically significant different with $p < 0.05$. In addition, there was no overlap of the interval WD between the two clones at 95% confidence interval (Fig. 2). This showed that the trees planted in similar environments, but

with different genetic backgrounds, led to different wood density [5-7], including the WD value of 6 year old *E. camaldulensis* of both clones that provided more than the value of *E. camaldulensis*, 4 year old in the eastern part of Thailand, which was 0.612 g/cm³ [39]. This is because when the trees got older, the density of the cell wall slightly increased while the lumen decreased [39-40]. In the same age case, the WD value of both clones that provided more than the WD value of *E. pilularis* and *E. cloeziana* in Australia with 0.497 and 0.612 g/cm³, respectively. [41]. It was because different basic genetics.

The leaf slenderness and thickness

Table 8 shows that A5 and D1 clones had the leaf slenderness of 1.18 and 1.19, respectively. The leaf slenderness of A5 was slightly lower than D1 and the average of leaf slenderness of the two clones was not statistically significantly different with a *p*-value of more than 0.05 (Table 10). In addition, there was no overlap of the interval of leaf slenderness between the two clones at 95% confidence interval (Fig. 3). As the leaves of A5 had a clumped distribution along the branches, while the leaves of D1 had regular distribution along branches, the leaf slenderness was an attribute that would promote leaves of A5 to be more penetrated

by the light. This complies with the report of Konôpka et al. (2016) [42], which specified the capability of leaves for light capture not only depended on the leaf surface area or leaf width but also depended on other factors, such as leaf plasticity.

Table 7. Statistical results for difference of the average of WD between A5 and D1 by Two-Sample T-Test.

Item	A5	D1
Mean of WD (g/m ³)	0.7364	0.6345
SD	0.0327	0.0723
SE Mean	0.0120	0.0270
Estimate for difference	0.101961	
95% Confidence interval for difference	0.033553, 0.170369	
<i>p</i> -value	0.009	

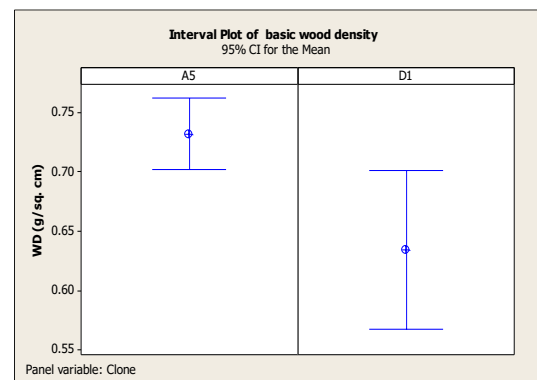


Fig. 2. The interval plot of basic wood density of 6-year old A5 and D1 at the 95% confidence interval.

Table 8. Statistical value of leaf width, length, surface area and slenderness of A5 and D1.

Statistical value	Width (cm)	Length (cm)		Leaf surface area (cm ²)		Square area (cm ²)		Leaf surface area: square area		Slenderness
		base	apex	base	apex	base	apex	base	apex	
Clone A5										
mean	3.31	3.99	9.80	9.55	19.89	13.26	32.32	0.72	0.62	1.18
SD	0.22	0.53	1.43	1.64	1.76	2.37	4.23	0.07	0.06	0.17
min	3.07	3.29	8.06	7.67	17.77	10.73	27.50	0.62	0.51	1.01
max	3.78	4.76	12.06	11.52	22.11	17.98	40.34	0.84	0.71	1.46
Clone D1										
mean	3.34	3.78	10.54	8.87	20.51	12.46	34.18	0.71	0.60	1.19
SD	0.55	0.51	2.07	1.27	1.27	1.68	2.10	0.05	0.04	0.13

Table 8. Statistical value of leaf width, length, surface area and slenderness of A5 and D1. (Continued)

Statistical value	Width (cm)	Length (cm)		Leaf surface area (cm ²)		Square area (cm ²)		Leaf surface area: square area		Slenderness
		base	apex	base	apex	base	apex	base	apex	
min	2.21	2.81	7.65	6.99	18.54	9.34	31.11	0.58	0.52	0.92
max	4.19	4.39	15.09	10.55	22.74	14.44	37.84	0.78	0.65	1.38

Table 9 shows that the average SLW of A5 and D1 were 0.0135 and 0.0120 g/cm² respectively and the SLW between two clones were statistically significantly different with a *p*-value less than 0.05 (Table 10). In addition, the internal value of leaf thickness between the two clones was not overlapped at 95% confidence interval (Fig. 3). It was found that A5 had more leaf thickness than D1, a characteristic promoted by its photosynthesis capacity, because the greater the leaf thickness, the more chlorophyll density per area [10, 14-16].

Table 9. Statistical value of leaf surface area, dry weight and specific leaf weight sampling from Clone A5 and Clone D1.

Item	Mean	St Dev	Min-Max
Clone A5			
LSA (cm ²)	573.63	60.38	498.36-665.23
LDW (g)	7.70	0.90	6.38-9.21
SLW (g/cm ²)	0.0135	0.0001	0.0122-0.0151
Clone D1			
LSA (cm ²)	705.77	39.23	640.59-747.10
LDW (g)	8.44	0.50	7.52-9.18
SLW (g/m ²)	0.0120	0.0001	0.0105-0.0136

note: LSA was Leaf surface area. LDW was Leaf dry weight. SLW was Specific leaf weight.

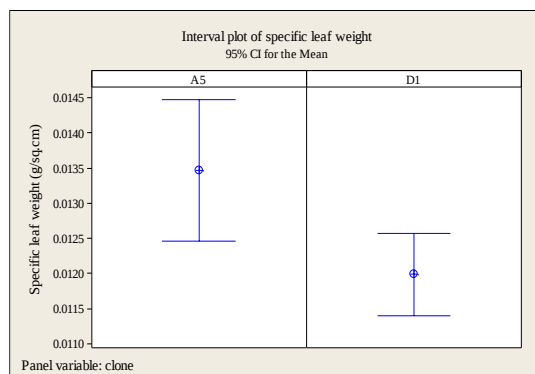
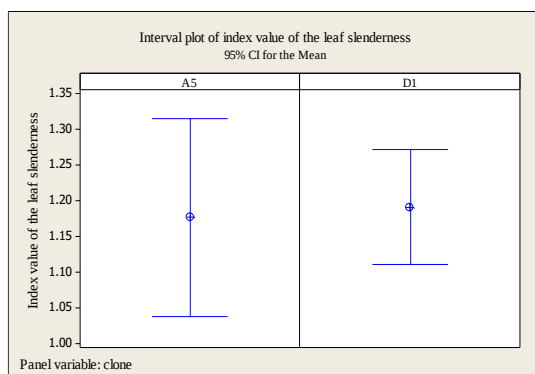
**Fig. 3.** The interval plot of the leaf slenderness and the specific leaf weight of 6year old A5 and D1 at the 95% confidence interval.

Table 10. Statistical analysis for difference of leaf slenderness and specific leaf weight between A5 and D1 by Two-Sample T-Test.

Item	A5	D1
Mean of Slenderness	1.78	1.19
SD	0.17	0.13
SE Mean	0.059	0.037
Estimate for difference	-0.0145	
95% Confidence interval for difference	-0.1657, 0.1366	
p-value	0.838	
Mean of SLW (g/m ²)	0.0135	0.0120
SD	0.0012	0.0009
SE Mean	0.0004	0.0003
Estimate for difference	0.0015	
95% Confidence interval for difference	0.0004, 0.0026	
p-value	0.012	

Conclusion

The A5 clone had the higher H/D ratio and less width of crown cover than the D1 clone, including the stem volume of A5, which was less than D1 (equaled 56.36 and 61.86 m³/ha respectively), but provided more dry mass of stem than D1 (30.95 and 28.40 ton/ha, respectively) because of the difference of WD. Moreover, the leaf slenderness of A5 and D1 were not different but the leaf thickness of both clones had differences in leaf thickness, of which A5 was more than D1 (0.0135 and 0.0120 g/m², respectively). In addition, the results indicated that having different basic genetics at a variety of levels could show characteristics of basic wood density and leaf thickness (0.7364 and 0.6345 g/cm³, respectively). Therefore, it implied that the A5 clone could be planted with more stand density than the D1 clone without competitive trees. Some morphology in this study could be used further to improve the genetic Eucalyptus. For selecting the suitable silviculture practices, a plantation owner needs to understand fully some morphology of trees which promote mass and growth.

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