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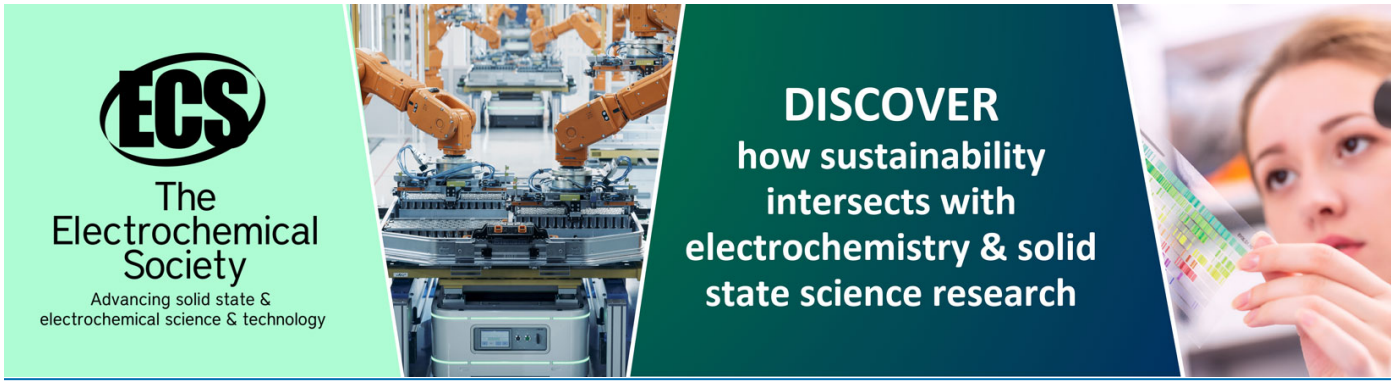

Morphological variation in the interspecific hybrid of *Acacia* (*Acacia mangium* × *A. auriculiformis*)

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Abstract. *Acacia* hybrid is an interspecific hybrid resulting from crossing between *Acacia mangium* and *A. auriculiformis* either naturally or artificially. This study aimed to determine the morphological variation of artificial *Acacia* hybrid, obtained by full-diallel control crossing. Observed sample trees were clones at the clonal test, established in 2011 in Wonogiri, Central Java. Tree samples were taken by selecting clones categorized into the superior, intermediate, and inferior clones based on their growth at one year of age, with three different clones in each category. Parameters to be observed were morphological characters including quantitative (tree height, diameter breast height, bole length, phyllode length and phyllode width) and qualitative (stem form, stem straightness, bark type, bark color, phyllode shape, phyllode apex shape, conspicuousness of the main vein, and petiole color). The data quantitative and qualitative were then scored and analyzed using NCSS series 12 software for cluster analysis. The results showed that morphological characters of *Acacia* hybrid clones varied across the tree categories. Furthermore, the dendrogram showed that the characters of *Acacia* hybrid grouped into two clusters: Cluster A dominated by superior and most intermediate clones, and Cluster B dominated by inferior clones. The study result implies the use of morphological characters of *Acacia* hybrid to select the hybrid vigor in a breeding program.

1. Introduction

An interspecific hybrid is a hybrid that results from crosses between the two different species in the same genus [1-4]. The interspecific hybrid may occur when two species have optimum genetic distance avoiding the internal barriers that prevent the genetic interchange between two species [3]. *Acacia* hybrid is an interspecific hybrid resulting from crossing between *Acacia mangium* and *A. auriculiformis*. The natural *Acacia* hybrid was found and reported firstly in Sabah and Ulu Sedili, Johor, Malaysia. The growth was vigorous as *A. mangium*, a better branching and lighter color bark than *A. mangium*, but inherited some poor forms of *A. auriculiformis* [5]. It was reported that the *Acacia* hybrid is better than *A. auriculiformis* on stem straightness and stem circularity with intermediate physical and mechanical wood properties between the two parent species [2].

Morphologically, the hybrid trees can be easily distinguished from their parents when the hybrid had intermediate features between the two parental species [1], such as a hybrid of *Pinus coulteri* × *P. jeffreyi* [2], *Eucalyptus urophylla* × *E. grandis* [6]. Following the Mendell Laws, the morphological characters of first-generation interspecific monohybrid are intermediate between the parental trees, showing a

combination of phenotypes representing genetically dominant from each parent [1, 7]. Uniformity of morphology on first-generation hybrid offspring was strongly dependent on the pure line of parent trees. However, sometimes it varies due to the presence of polygenes that govern the same characters and influence an environment or sites [8]. In *Acacia* hybrid, a study on the morphological similarity between the hybrid offspring and the parent species for the leaf, stem, and root anatomy showed that not all the hybrids have the intermediate characters of the parental trees [9]. It revealed that the character's appearance of the first-generation hybrid seems to depend on the interaction type between the parental genes, which show a dominant expressed [8, 10, 11].

The phenotype appearance variation of hybrid, either quantitative or qualitative traits, is influenced by the interaction between genes and environment, which could be assessed and analyzed using tools and software [12, 13]. Therefore, observing the phenotypes of first-generation *Acacia* hybrid offspring is very important in a breeding program to obtain the hybrid vigor or superior hybrid. In addition, a morphological marker for selecting a superior hybrid is very useful to reduce the cost of identifying the superior hybrid compared to molecular markers. Many studies have reported the morphological characters of *Acacia* hybrids to differentiate with their parent tree [2, 4, 17], but still rare studies that observe the variation of morphological character within the *Acacia* hybrids.

The studies about the growth and other properties of *Acacia* hybrid are also reported [4, 6], but identification of the morphological characters on superior *Acacia* hybrid is still limited. Therefore, this study aims to observe morphological variation on first-generation *Acacia* hybrid offspring based on the quantitative and qualitative data in order to obtain the morphological characters as markers on superior *Acacia* hybrid. Determining the morphological marker of superior *Acacia* hybrid was based on scored quantitative and qualitative data analyzed by software NCSS series 12.

2. Material and Methods

2.1. Plants materials and site description

The observation of this study was done directly in the field, with the observed tree samples categorized into three groups based on tree height growth ranking at the age of 1 year, namely superior, intermediate and inferior clone group. Superior and intermediate clones were characterized by a single stem with average height ranging from 3-5 m and 1-3 m, respectively. In comparison, the inferior clones were characterized by multiple-stem to shrubby and under 1 m height. Furthermore, each group consisted of three different clones, namely Clone 44, Clone 25, and Clone 16 for the superior clone. In contrast, the intermediate clones are Clone 13, Clone 22, and Clone 28 and the last, the inferior clones are Clone 5, Clone 11, and Clone 31 with each group of three trees, and the total number was 27 sample trees.

The sample tree used in this study were *Acacia* hybrid (*A. mangium* × *A. auriculiformis*) clones tested in the clonal trial. The trial was established on 2011, with a spacing of 3 × 3 m at Kawasan Hutan Dengan Tujuan Khusus (KHDTK) of Alas Ketu, Wonogiri District, Central Java Province. The *Acacia* hybrids were interspecific hybrid from a full-diallel controlled crossed pollination between *A. mangium* as the female parent and *A. auriculiformis* as the male parent. The trial site's latitude (South) and longitude (East) are 7°32' and 110°41' respectively, with an altitude of 141 m asl. The climate was Type C based on Schmidt and Fergusson classification with the mean annual rainfall of 1878 mm year^{-1} and minimal-maximal temperature of 22.0°C-33.6°C. The type of soil is Vertisol which usually shows a deep cracking in the dry season and heavy clay in the rainy season.

2.2. Measurement and data analysis

The observed parameters covered the quantitative and qualitative characters. The quantitative parameters were total height, bole length, diameter at breast height (dbh), length and width of phyllode, while the qualitative was the morphological of stem-straightness, type and color of bark, and shape of phyllode and its apex, and the anthocyanin content on the petiole. The morphological characters were determined according to the Testing Guidelines for *Acacia* species [13].

Total tree height and bole length were measured using a 20-meter pole, while the dbh was measured using phi-band. The barks were observed on each sample tree from three different face directions of 1.3

m stem height. The Color Chart Standard (RHS color chart) was used to determine the color of bark. The phyllode (leaf) from each tree was observed using three branches with each of three leaves. Some of the sampled phyllodes were then prepared for the herbarium.

The collected data were then analyzed using two methods of analysis variance and clustering analysis. Analysis variance was made to determine the statistical differences of the quantitative parameter. While the clustering analysis was made using scored data transformed from both quantitative and qualitative data [14]. Clustering analysis was run using the NCSS 12 software to obtain a dendrogram informing the clone's cluster based on the observed characters [15].

3. Results and Discussion

3.1. Quantitative parameters

The measured quantitative parameters of *Acacia* hybrid (*A. mangium* × *A. auriculiformis*) clones are presented in Table 1. Mean of total height, bole length, dbh, phyllode length, and phyllode width varied, ranging from 7.67-16 m, 1.21-7.73 m, 9.3-23 cm, 14.10-19.22 cm and 3.84-5.19 cm, respectively. Based on these quantitative data, the clones were then classified into three categories: superior clones (Clone 44, Clone 25, Clone 16), intermediate clones (Clone 13, Clone 22, Clone 28), and inferior clones (Clone 5, Clone 11, Clone 31). The average height of superior, intermediate, and inferior clones was 15.56 m, 11.48 m, and 10.23 m, respectively, while the dbh was 21.11 cm for superior and 15.60 cm for intermediate and 10.26 cm for inferior. The height and diameter observed in this study are similar to the description of *Acacia* hybrid trees reported by [16] but higher than the *Acacia* hybrids grown in Vietnam [4]. This indicated that growth was influenced by the site or environment, especially the climate, such as precipitation and daily temperature. Indonesia is a tropical country warmer than Vietnam, so the *Acacia* hybrid growth is better and faster.

Other quantitative parameters of phyllode's size showed that the largest and smallest phyllode was found on the superior and inferior clones, respectively (Table 1). While for the intermediate clones, the phyllode ranged between both the superior and inferior clones. The size of the phyllode in this study was similar to other studies reported by [17] and [16], which described that the length and width of the *Acacia* hybrid was around 15-20 cm and 4-6 cm, respectively. However, compared to the parental tree species, the average phyllode's length and width in this study's Acacia hybrid were intermediate, ranging between the *A. mangium* as female and *A. auriculiformis* as male. The average length was 21.39 cm for *A. mangium* [18] and 10 cm for *A. auriculiformis* [19], while the average width was 7.7-15 cm and 2-5 cm for *A. mangium* and *A. auriculiformis*, respectively [20, 18].

Analysis of variance showed that all quantitative parameters measured in this study were significantly different ($p<0.01$) among the nine clones, as presented in Table 2. It indicated that the three sets of categories have clearly represented the three differences of the clone superiority based on the observed quantitative parameters. Therefore, this information would be useful to determine the morphological variation of *Acacia* hybrid, which is important in the genetic selection in further breeding programs. In comparison, quantitative parameters such as height and diameter are very important to assess the stand productivity when the clones are planted on the mass scale of plantation to supply raw materials for wood-based industry. The growth characteristics of height and diameter on Acacia hybrids are one of the main characters to select the superior *Acacia* hybrid through the breeding program. The result of the analysis variance also proved that growth on *Acacia* hybrid is influenced by the environment and the genetic. Therefore, the hybridization between improved parent trees (co-improve method) could increase the probability of obtaining the superior hybrid [9].

Table 1. The quantitative parameters of *Acacia* hybrid (*A. mangium* × *A. auriculiformis*) clones in Wonogiri, Central Java.

Quantitative parameters	Superior			Intermediate			Inferior		
	Clone 44	Clone 25	Clone 16	Clone 13	Clone 22	Clone 28	Clone 5	Clone 11	Clone 31
Height (m)*	15.67	15.00	16.00	8.27	13.33	12.87	7.67	11.03	12.0
Bole length (m)*	3.73	7.73	3.23	2.15	3.48	1.66	1.21	1.29	1.07
Diameter (dbh) (cm)*	23.00	17.81	22.53	9.93	13.97	11.83	9.3	10.77	10.53
Phyllode length (cm)*	19.22	15.83	15.11	14.02	16.50	16.51	14.17	15.13	14.10
Phyllode width (cm)*	4.92	4.34	5.19	4.87	3.84	4.23	3.91	4.70	4.10

Remark: *mean value

Table 2. Analysis variance of quantitative parameters on *Acacia* hybrids (*A. mangium* × *A. auriculiformis*) clones in Wonogiri, Central Java.

	Degree of freedom	Mean square	Probability
Height	8	27.29	<0.0001**
Bole length	8	13.25	0.0002**
Diameter (dbh)	8	87.38	<0.0001**
Phyllode length	8	12.11	0.0022**
Phyllode width	8	0.68	0.0001**

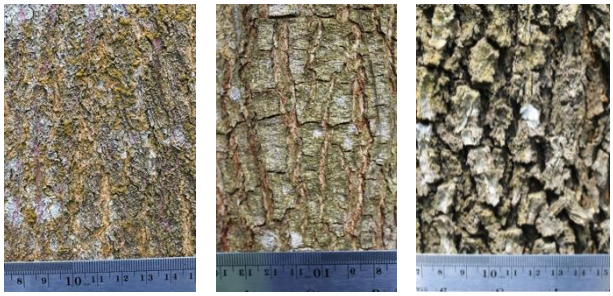
Remark: **significantly different on $\alpha = 0.01$

3.2. Qualitative parameters

The observed qualitative parameter is presented in Table 3. The form and straightness of the stems on the superior *Acacia* hybrid clones were straight monopodial. In contrast, the inferior clones showed crooked sympodial stems, while the intermediate clones consisted of straight monopodial and crooked sympodial. The morphological characters of bark among the superior and intermediate clones varied both in color and type, while the inferior clones were uniform. The bark color of the superior clones varied from brown to dark brown, with the type of bark smooth, fissured and cracked (Figure 1). Meanwhile, the bark color of the inferior was smooth greyish brown bark, while for the intermediate clones was brown to greyish brown with the smooth to cracked bark type.

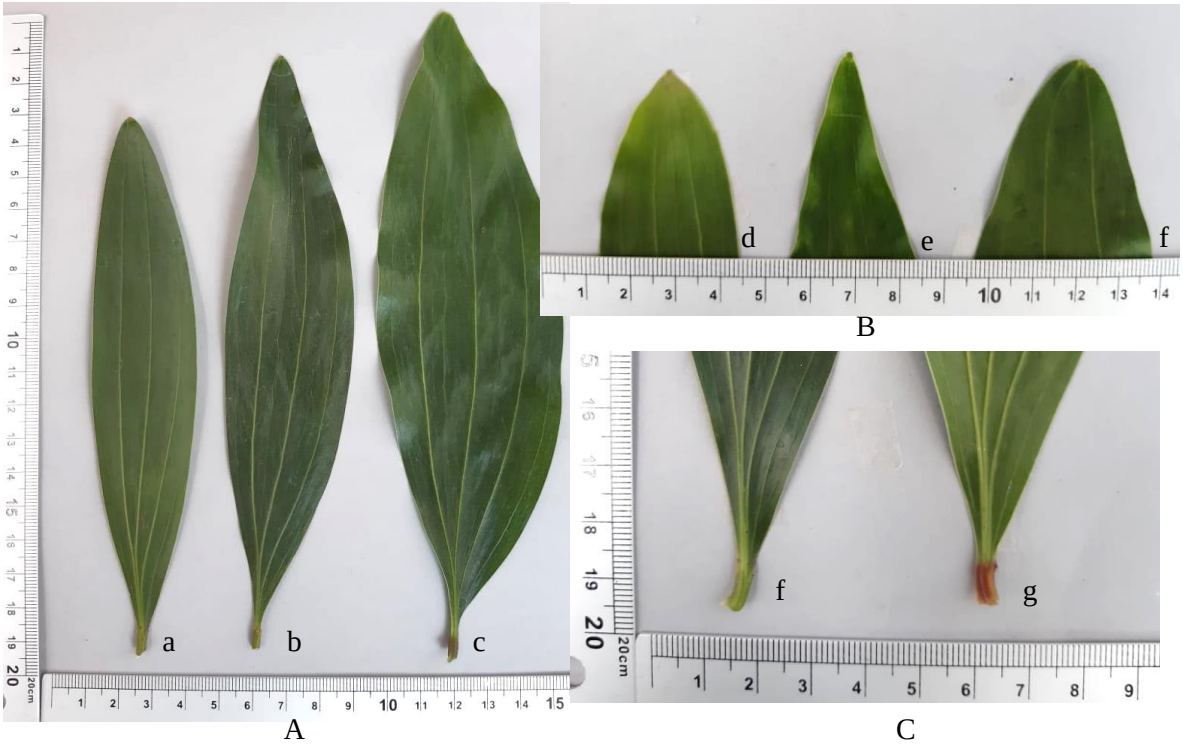
Table 3. The qualitative parameters of *Acacia* hybrid (*A. mangium* × *A. auriculiformis*) clones in Wonogiri, Central Java.

Qualitative parameters	Superior			Intermediate			Inferior		
	Clone 44	Clone 25	Clone 16	Clone 13	Clone 22	Clone 28	Clone 5	Clone 11	Clone 31
Stem									
form	monopodial	monopodial	monopodial	monopodial	monopodial	sympodial	sympodial	sympodial	sympodial
straightness	straight	straight	straight	straight	straight	crooked	crooked	crooked	crooked
Bark									
color	Dark brown	Dark brown	Reddish-brown	Brown	Greyish brown	Brown	Greyish brown	Greyish brown	Greyish brown
type	Cracked	Fissured	Cracked	Cracked	Smooth	Cracked	Smooth	Smooth	Smooth
Phyllode									
shape	Slightly convex	Slightly convex	Slightly convex	Slightly convex	Slightly convex	Slightly convex	Slightly convex	Straight	Straight
apex	Blunt	Blunt	Blunt	Acuminate	Acute	Acuminate	Acute	Acute	Acute
main vein conspicuousness	Medium	Strong	Medium	Weak	Medium	Weak	Medium	Weak	Weak
Petiole									
anthocyanin intensity	Present Strong	Present Weak	Present Strong	Present Medium	Absent -	Absent -	Absent -	Absent -	Absent -



a b c

Figure 1. Bark type variation on *Acacia* hybrid (*A. mangium* × *A. auriculiformis*) clones in Wonogiri, Central Java: smooth (a), fissured (b) and cracked (c).



A B C

Figure 2. A) Phyllode shape variation on *Acacia* hybrid (*A. mangium* × *A. auriculiformis*): straight (a) and slightly convex (b) and convex (c), B) Phyllode's apex shape: acuminate (d), acute (e), blunt (f) and C) anthocyanin intensity on petiole: absent of anthocyanin (f) and strong of anthocyanin (g).

All clones showed an almost uniform phyllode shape (slightly convex), except on two inferior clones (clone 11 and 31), which was straight (Figure 2A). The phyllode apex on superior *Acacia* hybrids are uniform (blunt) but varied from acute to acuminate on the intermediate and inferiors clones (Figure 2B). Meanwhile, the conspicuousness of leaf veins varied from medium to strong for the superior clones and weak to medium for the other clones categories. Finally, the anthocyanin content in petiole was found on superior and intermediate clones with weak to strong intensity, but none on the inferior clones (Figure 2C).

The qualitative traits of superior *Acacia* hybrid clones in this study were almost similar to other studies in Vietnam, which reported that *Acacia* hybrid at two years of age had a monopodial straight stem, brown fissured bark, and phyllode length and width of 15-10 cm and 4-6 cm, respectively [21].

However, compared to the parental species trees, the superior *Acacia* hybrid clones were closer to *A. mangium*, a female tree characterized by a straight stem with fissured brownish dark bark and phyllode's size almost 25 cm long and 10 cm wide with the conspicuous main veins [22]. In contrast, the inferior clones were closer to *A. auriculiformis* as a species of male tree that reported a crooked sympodial stem with the smooth greyish bark and straight-curved phyllode of 10-16 cm long and 1-3 cm wide and inconspicuous main veins [23, 19]. Meanwhile, the intermediate clones were dominated by clones that showed similar features to the female trees of *A. mangium* (Clone 13, Clone 22) and a few like male trees of *A. auriculiformis* (Clone 28). Although this result showed that the qualitative characters of the *Acacia* hybrid tend not to be influenced by the environment, it was proved that the qualitative traits of the *Acacia* hybrid in Indonesia and Vietnam are almost similar. Meanwhile, the qualitative characters of *Acacia* hybrid vary compared to their both species parent trees, which mostly have intermediate characteristics between their parent trees and some tendencies similar to the female or male tree.

3.3. Dendrogram

The dendrogram as a result of cluster analysis is presented in Figure 4. Two main clusters were formed from 27 sample trees based on their observed quantitative and qualitative parameters, namely A and B clusters (Figure 4A). Cluster A was a grouping of 12 trees consisting of nine trees from the superior clones category and three from intermediate clones. The remaining 15 trees were grouped into cluster B. Cluster A was grouped by the traits of the monopodial straight stem, fissured or cracked stem bark with the color dominated by dark brown, and presence of anthocyanin on the petiole. The growth of trees based on height, dbh, and bole length on Cluster A was better than cluster B and had a bigger phyllode's size (length and width). Meanwhile, Cluster B was grouped by the traits of sympodial stem form, greyish brown smooth bark, and no anthocyanin on the petiole. However, the tree growth was poorer than Cluster A, with a smaller phyllode size.

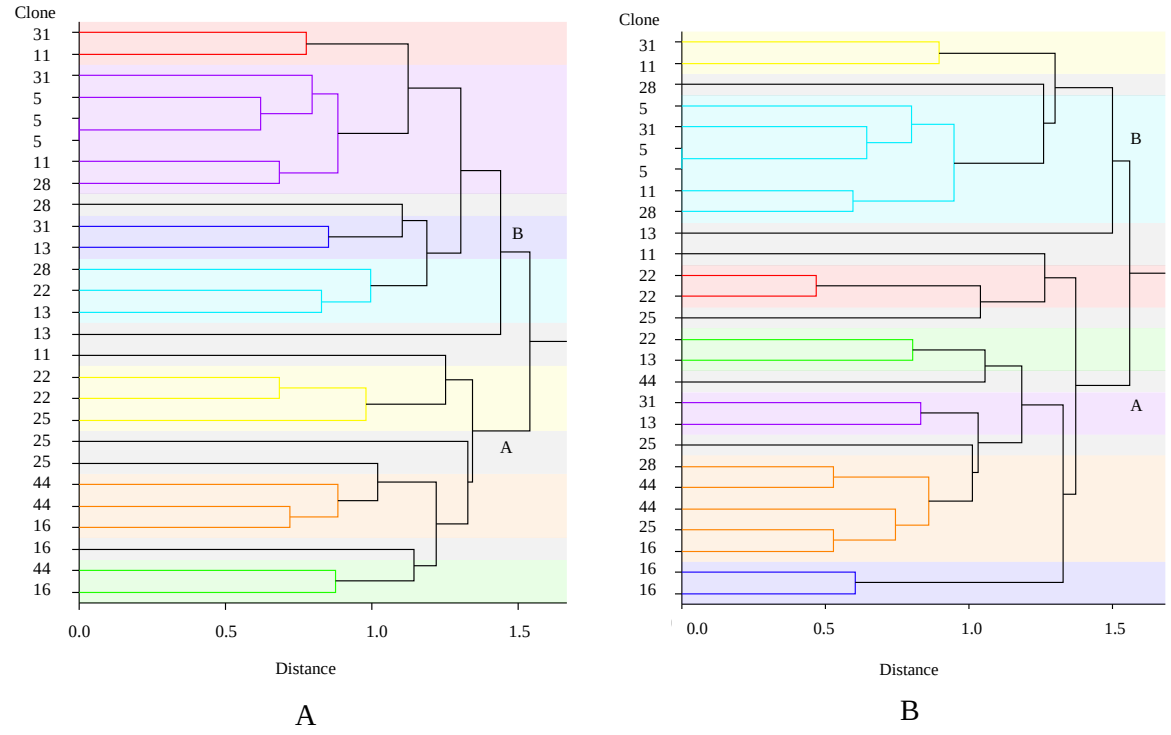


Figure 3. Clustering of *Acacia* hybrids clone based on quantitative and qualitative characters (A) and based on qualitative characters only (B).

Figure 4B shows two main clusters (Cluster A and B) grouped based only on qualitative characters. After the quantitative characters were excluded, Cluster A, which previously consisted of 12 trees (superior clones and a small portion of intermediate clones), changed to 17 trees that were nine from superior clones, and eight trees mostly from the intermediate clones. Otherwise, Cluster B consisted of 10 trees (inferior clones and a small portion of intermediate clones) which previously consisted of 15 trees (inferior clones and a large portion of intermediate clones). The intermediate clones have qualitative characters closer to the superior clones, but their growth is similar to the inferior ones. In this paper, the cluster analysis based on the quantitative character only did not analyze due to the aim of this study which focused on the morphological characters of *Acacia* hybrid. Therefore, the quantitative characters have been analyzed using the analysis of variance.

Based on the previous study [9, 19, 24], the Figures 4A and 4B above also indicated that the superior *Acacia* hybrids clones have a higher level of similarity to the female parent tree than the male tree based on their growth (quantitative characteristics) and morphological characteristics (qualitative characteristics). In contrast, the inferior clones tend to close the male tree. The intermediate *Acacia* hybrid clones were morphologically more similar to *A. mangium* as a female tree but had growth traits closer to *A. auriculiformis* as a species male tree.

This study suggested that the female parent tree influenced the *Acacia* hybrid's character more than the male tree. Therefore, it caused the hybrid offspring to be morphologically similar to the female parent tree through the maternal effect or maternal inheritance [25]. Other studies on the relationship between *Acacia* hybrid and the parent trees based on the leaf, stem, and root anatomy reported that female trees influence more hybrids than male trees [9]. The study on *Eucalyptus* hybrid also reported that *E. gilii* × *E. socialis* hybrid has morphological characters closer to the maternal parent [26].

3.4. Implication for Acacia hybrid breeding

Hybrid morphological characters are easy to recognize when the parent trees have clearly different characters [25], particularly on the interspecific hybrids. In line with the result of this study that the *Acacia* hybrids clones obtained from the control cross between *A. mangium* and *A. auriculiformis*, it could be recognized well either based on the qualitative and quantitative characteristics. Furthermore, this study showed that the characteristics of the inter-specific *Acacia* hybrid were significantly varied among the superior, intermediate, and inferior hybrid categories based on the quantitative and qualitative parameters.

The *Acacia* hybrid breeding program aims to obtain the *Acacia* hybrid, which is superior in growth and superior on wood quality, resistant to pest/disease, and adaptive to marginal sites [26]. The result of this study could be implied on the *Acacia* hybrid breeding program for pre-screening to observe other *Acacia* hybrid characteristics not included yet in this study, such as wood properties, pest/disease resistance, and adaptability to marginal. The tree growth character is not the only one that is necessary to be improved to obtain the superior *Acacia* hybrid. However, wood quality, tolerance to attacked pest/disease, and adaptability to the marginal site also needed to be improved simultaneously. Therefore, a tandem selection must be applied to its breeding process, which sometimes needs pre-screening for time efficiency to further selection to achieve the superior *Acacia* hybrid. The qualitative characters of the plants are beneficial for this pre-screening process due to their easily recognized characters. After pre-screening, the growth selection could be made, followed by the wood properties and other characters selection needed.

4. Conclusion

The result showed that the quantitative or qualitative character of *Acacia* hybrids varied. The intermediate *Acacia* hybrid clones and have a higher similarity to the superior clones or female parent tree than inferior clones or the male tree. It was proved that the maternal effect occurred in the *Acacia* hybrid either at the clone level (55.6%) or the individual level (69.9%).

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Acknowledgment
We gratefully acknowledged the Faculty of Forestry, Gadjah Mada University, for sample observation and the Acacia and Eucalyptus tree improvement team at Center for Forest Biotechnology and Tree Improvement for their support in establishing and evaluating the clonal test of *Acacia* hybrid in Wonogiri, Central Java.

Authors' contributions
All authors contributed equally to this work as the main contributor.