Relationship between *Acacia mangium* × *Acacia auriculiformis* hybrids and their parents based on leaf and root anatomy characteristics

S Sunarti*1, A Nirsatmanto1, I Permatasari2, Suharyanto2

1Center for Forest Biotechnology and Tree Improvement Research, Jl. PalaganTentaraPelajar Km 15Purwobinangun, Pakem, Sleman, DI Yogyakarta, Indonesia,
2Faculty of Biology, GadjahMada University, Jl. Teknik Selatan, DI Yogyakarta, Indonesia.

Email:nartinirz@biotifor.or.id; narti_nirsatmanto@yahoo.com

**Abstract.** *Acacia mangium* × *Acacia auriculiformis* hybrids do not always perform intermediate features of both parents. A study was conducted to observe the similarity of *Acacia* hybrid progenies to their parents based on anatomical features of leaf and root. Leaf and root samples were taken from each three sampled trees of *Acacia* hybrid and parent species selected on three growth superiority classes: superior, intermediate and inferior. The anatomical features were observed on leaf and root tissue specimens prepared through free-hand and non-embedding technique. Similarity and relationship indexes were determined using cluster analysis. The similarity index between the superior hybrid and female tree was higher than that of the male tree with similarity index value of 88.45% and 87.60% respectively. Based on the phenogram analysis, the parent species were clustered into two clearly separated groups with the distance of 86%. The superior and intermediate classes of *Acacia* hybrids tended to cluster into female parent group. Superior *Acacia* hybrids could be potentially produced by selecting the superior female parents as mother.

1. Introduction

*Acacia* hybrid is an offspring resulted from crossing between *Acacia mangium* × *Acacia auriculiformis* in which its morphological characters areusually intermediate between those of parental species[1][2]. The morphological characteristics of *Acacia* hybrid vary greatly, some are intermediate [2] while the remaining looks like their parents[3]. Morphological characteristics of seedling leaves are already well recognized and have becormarkers for indentifying the *Acacia* hybrid in the nursery [2][4]. Morphological characters are very easily differentiated between hybrid and the two parents, but not yet known for their anatomical properties.
The anatomical properties of seedling leaves, stem and roots vary between the *Acacia* hybrids and their parents[4]. Based on anatomical structure of leaf, stem and root tissues of seedlings, similarities between the hybrids and parents were found at different levels. Hybrids of *Acacia* (*A. mangium* × *A. auriculiformis*) tend to be closer to mother tree with the similarity index up to 60% [6]. The anatomical structure on leaf, root and stem tissues of *Acacia* hybrid seedling might be strongly inherited from the mother trees which apparently set by a maternally controlled mechanism as on seed's shape and size of common bean [7] and *Arabidopsis thaliana* [8].

The Center for Forest Biotechnology and Tree Improvement (CFBTI) in collaboration with the Faculty of Biology, Gadjah Mada University continued to observe the anatomical properties of superior clones of *Acacia* hybrids compared to the parent trees. This paper presents the relationship between superior, intermediate and inferior *Acacia* hybrids and their parents based on anatomical structure of leaf and root tissue. The implication of the anatomical characteristics of the seedlings and trees as morphological markers for identifying the vigorous growth of the *Acacia* hybrids is also discussed.

2. Materials and Method

2.1. Plant materials

Samples of roots and leaves were collected from an *Acacia* hybrid Clonal Test in Wonogiri, Central Java which was established in 2011. Based on Smith and Fergusson climate classification, Wonogiri belongs to type C with an average annual rainfall of 1878 mm, an average daily temperature of 22°C-33.6°C, and altitude of 141 m. Its soil is classified as Vertisol [9]. Leaf and root samples were taken from both *A. mangium* and *A. auriculiformis* as parental species, and from *Acacia* hybrid trees as hybrid progenies which are categorized on the basis of the growth performance as superior (Clone 44, 16 and 25), intermediate (Clone 8) and inferior (Clone 11). Three trees were sampled for each clone and parent tree species to collect leaf and root for anatomical observation in the laboratory.

2.2. Methods
Samples of mature leaves were collected by picking up the leaves from each sample tree which were then cut it into 1×1 cm and put in the flacon bottle containing 70% of alcohol. Root samples were collected by cutting 1 cm of root approximately 1 cm in diameter, then put in a flacon bottle filled with 70% of alcohol. The samples were then sliced using a sharp and sterile razor to 20-30 micrometers thick and stained with 1% safranin dye. Stained slices were then put on the microscope slide, dropped with one or two of albumin and closed with the coverslip carefully then applied with the cutex around the lid [10]. Root and leaf cross-section slides were examined on light microscope with a 4 × 10 and 10 × 10 magnification. The qualitative and quantitative data were then collected for analysis from the cross-section slide observation. Quantitative data used to calculate the similarity index were classified using three scale scores (1=small, 2=intermediate and 3= big (Table 1).

Table 1. Scores of anatomical structure in leaves and roots

| No  | Leaf anatomical structure     | Score 1 | Score 2 | Score 3 |
|-----|------------------------------|---------|---------|---------|
| 1   | Cuticle (µm)                 | 2.02-2.81| 2.91-3.6| 3.7-5.18|
| 2   | Upper epidermis (µm)         | 5.01-6.29| 6.30-7.57| 7.60-10.13|
| 3   | Lower epidermis (µm)         | 5.76-6.76| 6.77-7.81| 7.82-9.91|
| 4   | Upper palisade (µm)          | 35.28-37.59| 38.59-39.9| 40-44.53|
| 5   | Lower palisade (µm)          | 36.25-37.97| 38.97-39.69| 40.69-43.13|
| 6   | Sponge tissue (µm)           | 95.78-108.64| 109.64-121.5| 122.5-147.22|
| 7   | Midrib vascular bundle (µm)  | 38.56-46.52| 47.52-54.48| 55.48-70.40|
| 8   | Lamina vascular bundle (µm)  | 43.14-49.62| 50.62-56.1| 57.1-69.06|
| 9   | Axis (µm)                    | 306.27-359.26| 360.26-412.25| 413.25-518.25|
| 10  | Mesophyll (µm)               | 119.11-148.63| 149.63-178.15| 179.15-237.19|

| No  | Root anatomical structure    | Score 1       | Score 2       | Score 3       |
|-----|------------------------------|---------------|---------------|---------------|
| 11  | Periderm (µm)                | 116.10-126.85| 127.85-137.6  | 138.6-159.1   |
| 12  | Cortex (µm)                  | 70.98-78.64   | 79.64-86.3    | 87.3-101.62   |
| 13  | Floem (µm)                   | 390.12-466.39| 467.39-542.66| 543.66-695.19|
| 14  | Metaxylem diameter (µm)      | 107.29-126.58| 127.58-145.87| 146.87-184.43|
| 15  | Metaxylem density (unit µm²) | 41-53         | 54-65         | 66-89         |

Notes: score 1= small, 2= intermediate, 3= big

2.3. Statistical analysis

Operational Table Unit (OTU) was structured to classify the groups of individuals showing a closer relationship based on the anatomical characteristics. Cluster analysis was carried out using Multi Variate Statistical Package (MVSP) software. The similarity indexes between hybrid
progenies (A. mangium × A. auriculiformis) and parentswere calculated using Unweighted Pair Group Method (UPGMA) [11][12]. Similarity indexes are expressed in percentages (0-100%).

3. Result and Discussion

The cuticle layer, epidermal structure, location of palisade, vascular bundle type and stomata type of the leaf of Acacia hybrid and parent trees were uniform, but varied in shape of epidermal cell, midrib axis type, midrib vascular bundle size (Table 2). The shape of epidermal cells on A. auriculiformis and inferior Acacia hybrids was polygonal while the others were round. The type of midrib vascular bundle in A. auriculiformis and all Acacia hybrids protruded, except the poor hybrid. The protruding midrib vascular bundle in A. mangium, superior and intermediate Acacia hybrid might cause their leaves stiffer than A. auriculiformis and inferior Acacia hybrid. The size of midrib vascular bundle in A. auriculiformis and inferior Acacia hybrids was small (score 1), while the remainder was high (score 3). It seems that the leaf anatomical characters of inferior hybrids were influenced more by female than male parent, whereas those of intermediate and superior hybrids were less affected by female parent.

Table 2. Qualitative description of leaf anatomy in a 6-year-old Acacia hybrid in Clonal Test, in Wonogiri, Central Java.

| Characteristics       | Am | Aa | Clone 44 | Clone 25 | Clone 16 | Clone 8 | Clone 11 |
|-----------------------|----|----|----------|----------|----------|---------|----------|
| Cuticle layer         | Present | Present | Present | Present | Present | Present | Present |
| Epidermal shape       | Round | Polygonal | Round | Round | Round | Round | Polygonal |
| Epidermal structure   | Single layer | Single layer | Single layer | Single layer | Single layer | Single layer | Single layer |
| Palisade location     | Isobilateral | Isobilateral | Isobilateral | Isobilateral | Isobilateral | Isobilateral | Isobilateral |
| Midrib axis type      | Protruding | Not Protruding | Protruding | Protruding | Protruding | Protruding | Not Protruding |
| Vascular bundle size  | Big | Big | Big | Big | Big | Big | Big |
| Vascular bundle type  | Collateral open | Collateral open | Collateral open | Collateral open | Collateral open | Collateral open | Collateral open |
| Stomata type          | Amfistomatik | Amfistomatik | Amfistomatik | Amfistomatik | Amfistomatik | Amfistomatik | Amfistomatik |

Note: Am = A. mangium, Aa = A. auriculiformis

The size of cells which composed of the leaves of Acacia hybrids and the parents tree was varied (Table 3). Generally the cell size of Acacia hybrids was intermediate between that of both parents, as reported by Kha [2] who mentioned that the characters on the first generation of Acacia
hybrid are usually intermediate between those of parental species. The size of cuticle, sponge, midrib and lamina vascular bundle cells of Acacia hybrid was also varied and significantly differed between the clones (p<0.001). The female trees (*A. mangium*) had thinner cuticles than *A. auriculiformis*, while the hybrids were varied in thickness. Clone 25 had the thickest cuticle (Table 3). Based on the function of cuticle on the leaves, the superior hybrids might be more resistant to micro-organism attack and loss of water [13][14].

**Table 3.** Leaf anatomy measurement of 6-year-old *Acacia* hybrid in Clonal Test, Wonogiri, Central Java

| Characteristic                        | Am            | Aa            | Clone 44 | Clone 25 | Clone 16 | Clone 8 | Clone 11 |
|--------------------------------------|---------------|---------------|----------|----------|----------|---------|----------|
| Cuticle (µm)                         | 2.76±0.2      | 4.49±0.3      | 3.36±0.2 | 4.80±0.2 | 4.06±0.2 | 2.87±0.2 | 3.17±0.2 |
| Upper epidermis (µm)                 | 5.74±0.4      | 8.56±0.3      | 9.34±0.5 | 7.59±0.3 | 7.06±0.3 | 7.37±0.3 | 6.52±0.2 |
| Lower epidermis (µm)                 | 7.43±0.4      | 8.07±0.2      | 9.22±0.3 | 7.54±0.7 | 7.37±0.3 | 6.26±0.3 | 6.88±0.2 |
| Upper palisade (µm)                  | 38.27±0.7     | 42.94±1.4     | 36.83±1.0| 37.76±0.3| 40.07±0.7| 37.76±0.7| 40.21±0.4|
| Lower palisade (µm)                  | 38.66±0.6     | 41.59±1.3     | 39.14±0.7| 37.69±0.8| 38.67±0.4| 37.59±0.6| 41.07±0.8|
| Sponge tissue (µm)                   | 146.22±0.6    | 127.05±0.7    | 123.44±5.5| 118.91±2.9| 132.85±1.5| 106.30±3.6| 98.87±2.7|
| Midrib vascular bundle (µm)          | 53.71±1.6     | 43.65±2.0     | 68.17±1.6| 63.65±2.1| 65.86±3.1| 54.49±2.4| 40.85±1.5|
| Lamina vascular bundle (µm)          | 45.81±1.76    | 50.57±1.47    | 52.42±1.65| 53.65±2.1| 55.92±1.6| 65.05±1.73| 54.01±1.71|
| Axis (µm)                            | 514.97±3.10   | 392.35±2.58   | 495.77±4.9| 462.31±1.5| 493.07±2.8| 457.91±2.1| 307.9±1.36|
| Mesophyll (µm)                       | 223.53±3.10  | 234.82±2.54  | 211.79±1.8 | 21496±3.2 | 231.07±1.1 | 218.17±1.3 | 202.73±2.27 |

Note: Am = *A. mangium*, Aa = *A. auriculiformis*

This finding was similar to the previous study which observed leaf anatomical properties of *Acacia* hybrid in the nursery [5]. The properties of leaf anatomy, such as epidermis shape, type of midrib axis and size of vascular bundle might be used as markers to identify the hybrid superior of *A. mangium* x *A. auriculiformis*. The shape of epidermis was round with the protruding type of midrib axis and vascular budle of big size.
Figure 1. Leaf cross section on *Acaciamangium* (A), *Acacia auriculiformis* (B), superior *Acacia* hybrid (C,D), intermediate *Acacia* hybrid (E) and inferior *Acacia* hybrid (F), showing epidermis (a), cuticle layer (b), palisade tissue (c), sponge tissue (d), vascular bundle (e), cholenkim tissue (f), and axis (g).

Metaxylem, type of wall thickening, and vascular bundle in *Acacia* hybrid rootswere similar, except in intermediate hybrid in which the distribution of metaxylem was unequal and tended to be denser to the middle. The type of wall thickening in all observed trees was Oor ring type, while the type of vascular bundle was open collateral with the radial type of the development.
Table 4. Root anatomy measurement of 6-year-old *Acacia* hybrid in Clonal Test, Wonogiri, Central Java

| Parameter                  | Am             | Aa             | Clone 44 | Clone 25 | Clone 16 | Clone 8 | Clone 11 |
|----------------------------|----------------|----------------|----------|----------|----------|---------|----------|
| Periderm (µm)              | 123.17±1       | 154.48±2.5     | 118.26±1.3 | 120.12±0.9 | 119.15±1  | 123.49±1.5 | 132.89±1.6 |
| Cortex (µm)                | 79.50±0.8      | 99.12±2.7      | 77.20±1.5 | 76.64±1.3 | 77.41±1.1 | 80.28±1  | 72.57±0.6  |
| Floem (µm)                 | 455.23±1.8     | 545.59±3.1     | 662.76±2.1 | 692.17±1.7 | 580.96±5.1 | 569.98±4.2 | 396.96±5.4 |
| Metaxylem diameter (µm)    | 139.69±3.1     | 144.19±2.8     | 168.61±4.9 | 179.12±3  | 130.76±3.2 | 113.93±4.2 | 147.31±7.2  |
| Metaxylem density (unit µm³⁻²) | 44.67±3       | 68.33±2.6      | 55.67±2.6 | 78.00±2.29 | 62.00±2.29 | 75.00±3.46 | 84.00±3.9   |

Note: Am = *A. mangium*, Aa = *A. auriculiformis*

The cell size of anatomical structure of *Acacia* hybrid roots was significantly different between clones and parents tree (p<0.01). Generally the size of periderm, cortex, phloem, metaxylem diameter and density on *Acacia* hybrids tended to have no clear pattern whether it was intermediate between both parents tree or not (Table 5). This finding was similar to that of the previous study of anatomical properties of *Acacia* hybrids root in the nursery [5].
Figure 2. Root cross sections on Acaciaramgium (A), Acaciaauriculiformis (B), superior Acacia hybrid (C,D), intermediate Acacia hybrid (E, F) and inferior Acacia hybrid (G,H), showing an exodermis (a), cortex (b), endodermis (c), phloem (d), cambium (e), metaxylem (f) and pithrays (g) and branch root initiation (h)

Based on qualitative and quantitative descriptions standarized into OTU Table, the similarity index was assessed. The similarity index between the superior hybrid and female trees was higher than that of between the superior hybrid and the male tree with similarity index value of 88.45% and 87.60% respectively. Meanwhile for the intermediate hybrid, the similarity index between female and male tree was relatively balanced with the value of 83%. In contrast forinferior hybrid, its similarity index to male trees was higher than to the female trees with the value of 88.19% and 83.35% respectively. Based on the similarity index, the phenogram then configured to show the relationship between the hybrids and parents tree (Figure 3).

The phenogram showed that the hybrids and parents tree were clustered into two groups, A and B. The group A consisted of inferior hybrid (Clone 11) and the male tree (A. auriculiformis), while intermediate and superior hybrids were clustered into group B. In the group B, the hybrid was clustered into intermediate (clones 8 and 25) and superior (clones 44 and 16) hybrids. The
inferior hybrid was closer to the male than female parents, while the intermediate and superior hybrids tended to closer to the female parent. The similarities within the group A lied in number of metaxylem, cuticle thickness, upper and lower epidermal cells, epidermal cell shape, diameter of metaxylem, cortex and palisade thickness, while the similarities within the group B lied in thickness of vascular bundle and lamina, axis protuding, and the thickness of phloem and sponge tissues.

![Phenogram of Acaciamangium, Acacia auriculiformis and the hybrids based on similarity index of anatomical structure on leaf and root.](image)

Figure 3 Phenogram of Acacia mangium, Acacia auriculiformis and the hybrids based on similarity index of anatomical structure on leaf and root.

The superior hybrid tended to be closer to the female parent rather than the male parent. Apparently, it is due to an effect of the cytoplasmic inheritance partially or wholly [7][15][16]. In the cytoplasmic inheritance, female parent contributes the largest amount of cytoplasm to the zygote. Cytoplasm contains organelles with their DNA, including mitochondria and chloroplasts [8] [16]. These results imply that hybrid breeding strategy to obtain superior Acacia hybrid, particularly through the co-improvement method [9], would provide more advantages if A. mangium is used as female parent. Therefore breeding for improving the pure
parent tree species in Acacias is necessary to be continued to explore and obtain other potential genes relevant to the user need which could be further used in Acacia hybrid breeding program.

4. Conclusion

The superior and intermediate Acacia hybrids were grouped into one cluster with female parent, while the inferior hybrid was grouped into another cluster of male one. The leaf anatomical characters of superior Acacia hybrids differed on shape epidermis, type of midrib axis and the shape of vascular bundle which might be strongly influenced by the female tree genes rather than the male.

Acknowledgment

The authors greatly acknowledged The Faculty of Biology Gadjah Mada University for sample preparations and also the CFBTI team of Acacia and Eucalyptus tree improvement for their support on establishing the clonal test of Acacia hybrid.

References

[1] Rufelds CW. 1988. Acacia mangium, Acacia auriculiformis and hybrid A. mangium × A. auriculiformis) seedling morphology study. FRC Publication 41. Forest Research Center Publication. Malaysia.

[2] Kha LD. 2001. Studies on the Use of Natural Hybrids Between Acacia mangium and Acacia auriculiformis in Vietnam. Agriculture Publishing House. Hanoi.

[3] Sunarti S, Adyantara VD. 2014. Variasi morfologi dan kualitas benih akasia hibrida alamai generasi kedua (F-2) Dalam: Widyatmoko AYPBC, Nirsatmanto A, Baskorowati L, Mahfudz, Prabawa S (eds). Prosiding: Seminar Nasional Benih Unggul untuk Hutan Tanaman, Restorasi Ekosistem, dan Antisipasi Perubahan Iklim. Yogyakarta.

[4] Gan E, Sim BL. 1991. Nursery identification of hybrid seedlings in open plots. In: Carron LT, Aken KM (eds). Proceeding: Breeding Technologies for Tropical Acacias. Canberra.

[5] Fitriana V. 2011. Perbandingan struktur anatomis antara Acacia mangium Willd. (Pedley), Acacia auriculiformis A. Cunn dan hibridnya (Acacia mangium × Acacia
auriculiformis). Undergraduate Thesis. Faculty of Biology. Universitas Gadjah Mada. Yogyakarta.

[6] Sunarti S, Fitriana V, Suharyanto. 2018. Tingkat kesamaan Acacia mangium, Acacia auriculiformis, dan hibridnya berdasarkan sifat anatomi akar, batang dan daun. JIK/JFS. 12(2):234-247.

[7] Singh J, Michelangeli JAC, Gezan SA, Lee H, Vallejos CE. 2017. Maternal effects on seed and seedling phenotypes in reciprocal F1 hybrids of the common bean (Phasaelus vulgaris L.). Front. Plant Sci. 8(42). doi:10.3389/fpls.2017.00042

[8] Tsukaya H. 2005. Leaf shape: genetic controls and environmental factors. Int. J. Dev. Biol. 49:547-555. doi:10.1387/ijdb.041921ht

[9] Sunarti S, Na’iem M, Hardiyanto EB, Indrioko S. 2013. Breeding strategy of Acacia hybrid (A. mangium × A. auriculiformis) to increase forest plantation productivity in Indonesia. J Man Hut Trop 19(2):128-137. doi: https://doi.org/10.7226/jtfm.19.2.128

[10] Sas JE. 1958. Botanical Microtechnique. The Iowa State University Press. Iowa

[11] Romesburg HC 1984 Cluster analysis for researchers. Lifetime Learning Publication. Belmont, California.

[12] Sokal RR, Sneath PHA. 1963. Principle of Numerical Taxonomy. WH. Freeman & Co. New York.

[13] Liu Y, Cui H, Zhang Q, Sodmergen. 2004. Plant Phys. 136(1):2762-2770. doi: https://doi.org/10.1104/pp.104.04829

[14] Rost TL. Barbour MG, Stocking CR, Murphy TM. 2006. Plant Biology. Thomson Brooks/Cole. US.

[15] Suryo. 2005. Sitogenetika. Gadjah Mada University Press. Yogyakarta.

[16] Janicki DJ. 1953. Cytoplasmic Inheritance in Plants. Bios. 24(4):89-97.