



The Diversity of *Mangifera indica* Cultivars in Subang West Java Based on Morphological and Anatomical Characteristics

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DOI: 10.15294/biosaintifika.v9i1.8780

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History Article

Received 21 February 2016

Approved 16 March 2017

Published 1 April 2017

Keywords

anatomy; morphology;
mango; taxonomy; Subang

Abstract

The wide distribution and high species diversity produce large variation of its morphological, anatomical and genetic characteristics of local mangoes. Nowadays, most mango classification system is based on the generative character while the vegetative character is neglected. The purpose of this study is to develop foundation of vegetative character based on the similarity degree of mango cultivars based on the leaves' anatomy and morphology. In this study, 21 mango cultivars were found in Subang, West Java. Leave samples were collected from the field and soaked in 70% alcohol. Leaf anatomy preparats were made by whole-mount method and paraffin. The results showed a high similarity on all samples. There were three variations of vascular tissues on petioles, i.e. U-shaped (plano-convex), U-lobed (highly convex) and circular with the crystal type of druse, small cubes, and prismatic. All cultivars have actinocytic stomata and hypostomatic leaves. It could be concluded that stomatal density, size and index are less powerful to be applied as characters to identify cultivars of mangoes. However the presence of oxalate crystals can be used to classify mango cultivars in Subang.

How to Cite

Cahyanto, T., Sopian, A., Efendi, M. & Kinasih, I. (2017). The Diversity of *Mangifera indica* Cultivars in Subang West Java Based on Morphological and Anatomical Characteristics. *Biosaintifika: Journal of Biology & Biology Education*, 9(1), 156-167.

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p-ISSN 2085-191X

e-ISSN 2338-7610

INTRODUCTION

Mango (*Mangifera indica* L.) is a tropical fruit plant that could potentially be developed as a national commodity due to its high diversity. In Indonesia, mango is the third largest commodity, after banana and orange. This plant is also one of popular garden plants in Indonesia (Widjaja et al., 2014) and some regions in Indonesia also use it as medicinal plants (Yuniati et al., 2010; Irawan et al., 2013).

The wide distribution and high diversity may cause intraspecific variation in mango. It indicates the presence of a variety of shapes, sizes, flavours, and leaves scattered throughout Indonesia which caused a lot of variety cultivars of mango (Sumiasri et al., 2006; Nilasari et al., 2013; Widjaja et al., 2014; Cahyanto et al., 2016). It is very important to know the family links of intraspecies diversity of mango to determine its scientific taxonomy.

Recently, morphological-based identification is still widely applied. However, morphological character is highly influenced by environmental changes Sharma et al. (2012) such as on mango cultivars of Gujarat, India which showed high petiolar variations. Another approach is done by applying generative characters (Pakpahan, 2012) which highly depend on the availability of fruits. Thus, both approaches could hinder the development of taxonomical data on local mango and it is necessary to develop another approach for the identification such as the application of anatomy characters. This approach had been reported by Sharma et al. (2012) on different cultivars and probably could be applied on Subang mango cultivars.

To develop the method, it is necessary to collect data of morphology, generative, and anatomy on various mango cultivars of Subang as the purpose of this study. In the future, this data could be applied as the basis of information for breeding programs of local mango cultivars.

METHODS

Mango cultivar samples were collected from three locations around Subang; Pamanukan, Subang and Cagak Street. Leaf samples were collected and stored in 70% alcohol to observe its morphological and anatomical structure. Leaf morphology characteristics (Table 1) were observed following descriptive methods for mango descriptors (IPGRI, 2006) and scored as multivariate data. Prior to observation of the stomata, preparats of cross-sectional anatomy were

produced by paraffin and whole mount method. Observations on stomata were done for stomatal shape, type, location, size, density and index. Stomatal density and index were calculated by this following equation.

$$\text{The density of stomata} = \frac{\Sigma \text{ stomata}}{\text{Wide field of view (mm}^2\text{)}}$$

$$\text{Stomata index} = \frac{\Sigma \text{ stomata} \times 100\%}{\Sigma \text{ stomata} + \Sigma \text{ epidermal cells}}$$

$$\text{Wide field of view} = \pi r^2$$

Description:

π : constant (3,14)

r : objective micrometer radius

Morphological and anatomical data was analysed by *Numerical Taxonomy System* (NTSys) PC version 21. The similarity coefficient was determined by *Simple Matching* (SM) while the cultivars was classified by *Unweight Pair Group with Arithmetical Average* (UPGMA). The results are presented as a dendrogram by using TREE (Rohlf, 1998).

RESULTS AND DISCUSSION

The morphological characteristics of the leaf blade of mango cultivars

A total of 21 cultivars mangoes had been found in Subang. Most common cultivars were Manalagi, Arumanis, Cengkir, and Madu Keraton. Cengkir and Arumanis were the leading commodity for Subang and Indramayu. On the other hand, rare cultivars of Subang were Bapang, Golek, Ompyong, Muncang, Lenggak, and Gori. In this study, Tropong, one of abundant cultivars in Subang, was not found. It is assumed that lack of size and height of fibrous content in the fruit reduced the willingness of farmers to cultivate it.

Observation on leaf characters proved difficult to completely distinguish all cultivars (Table 2). This method is applicable as the key character for some cultivars with distinctive leaf shape and size, such as the Apel cultivar which has oblong-shaped and smaller leaf compare to others. Variation in shape leaf bases (rounded, tapered and pointed) in individual trees also became obstacle to apply leaf morphological characters as key character. It seems that fruit characters would be better to be applied as key character for each cultivar which has a unique shape and fruit aroma (Fitmawati et al., 2009).

The stomatal density, size and index of mango cultivars

Paradermal observations showed that lea-

Table 1. The characters used in the grouping of mango cultivars

Characteristics	Parameter	Score
Leaf Form (LF)	Lancet	0
	Elongated	1
Leaf Length (LL)	Up to 20 cm	0
	More than 20 cm	1
Leaf Width (LW)	Up to 5 cm	0
	More than 5 cm	1
Petiole Length (PL)	Up to 5 cm	0
	More than 5 cm	1
Leaf Texture (LT)	Rigid	0
	Wiry	1
Form of Leaf Apex/Apex Folii (FLA)	Spiky	0
	Tapered	1
	Rounded	2
Form Leaf Basal/Basis Folii (FLB)	Spiky	0
	Tapered	1
	Rounded	2
Form of Leaf Margin/Margo Folii (FLM)	Average	0
	Serrated	1
Surface of Upper Leaves (SUL)	Slick	0
	Hairy	1
Surface of Down Leaves (SDL)	Slick	0
	Hairy	1

ves of all cultivars had actinocytic stomata (a stomata with circles of 4 to 12 guard cells that spread in a radius) located on the abaxial leaves (hypostomatictype) (Figure 1) (Fahn, 1995; Prabhakar, 2004; Ganocpichayagrai et al., 2016). This condition is common in plants to reduce transpiration rate from stomata (Pugnaire & Pardos, 1999; Evert, 2006).

There were variations on the density, size and index of stomata in all observed cultivars (Table 3). The highest stomatal density was found in Apel cultivars (700.6 stomata/mm²) while the lowest was in Golek cultivar (629.2 stomata/mm²). Stomatal density of all cultivars considered as high category based on study of Tambaru et al. (2011) which stated that stomatal density with less than 300 stomata/mm² is categorised as low criteria and the density of more than 500/mm² as high criteria. High stomatal density is associated with resistance to drought test as it maintains turgor during dry period (Perwati, 2009).

Bapang and Arumanis cultivars which was cultivated in three different areas (Pamanukan, Subang, and Cagak Street) showed variation in stomatal density and size. The density of stomata of mango cultivated in Subang and Pamanukan tend to be higher (661 to 680.2 stomata/mm² for both Bapang and Arumanis cultivars combined)

compared to that at Cagak Street (452 to 456 stomata/mm² for both Bapang and Arumanis cultivars combined). This variation is allegedly associated with environmental conditions, such as temperature, altitude, and humidity. The stomatal density is inversely proportional to the size of the stomata which means a leaf with higher stomata density will has smaller stomata as indicated in Table 3 (Perwati, 2009). In dry condition, much smaller stomata will provide rapid response to change in water regime in plant while at the same time its high density maximise diffusion of CO₂ for optimal photosynthesis (Aasamaa et al., 2001). High stomatal density is associated to higher stomata conductance and transpiration which is necessary for water and nutrient in xylem, while the smaller stomata allow better stomatal resistance and control during water stress in low attitude area such Subang and Pamanukan (Hetherington & Woodward, 2003).

Stomatal index shows the ratio between the number of stomata and epidermis cell combined. Thus, higher stomatal index produces when numbers of stomata is higher than numbers of epidermis cell (Qosim et al., 2007). This index relates to the changes in the area of stomata and epidermal cells. The highest stomatal index (45.5%) recorded in Gori cultivar while the lowest is in

Gincir cultivar (43.2%). As Bapang and Arumanis cultivars which were found in three locations, the highest stomata index recorded from cultivars were cultivated at Pamanukan (44.2-45.2%) and the lowest was at Cagak Street (41.9%).

The diversity of cultivars Mango based on petiolar anatomy

Petiolar anatomy observations on 21 cultivars of mango showed three variations of the carrier beam, such as forming the U letter (planoconvex), U-lobed (highly convex) and round (circular) (Table 4). These types were also found in eight cultivars of Gujarat, India (Sharma et al., 2012).

Like other members of Anacardiaceae, single and closed carrier network systems were found consistently in all cultivars. According to Schweingruber et al. (2000) this system is characterised by a single collateral file on petiole where phloem was found on the abaxial side.

The existence of oxalate crystals on the leaf stalk also can be used as a taxonomic cha-

racter. There are three types of oxalate crystals found in this study: druse, small cubes and prismatic (Table 4). Oxalate crystal itself is an excess inorganic material produced from physiological activities of plant (Evert, 2006).

Classification of mango cultivars based on leaves' anatomy and morphology

Based on morphological and anatomical data of leaves, similarity among cultivars was recorded between 68-94% and divided into three main groups (Figure 2). Group I consisted of Apel, Cengkir Gajah, and Gedong Kelapa, all was originated from Pamanukan, with similarity value of 83%. Group I was separated with other groups by leaf size characters.

Group II, with two subgroups had 80% similarity value characterised by oxalate prismatic crystals found in the leaf stalk. Subgroup IIA had a similarity value 85%, consisted of Bapang 1, Bapang 2, Gincir, Nanas, Cengkir Kebongan, Golek, TO, Gidang, Muncang, and lenggak. Subgroup IIB with similarity value of 84.5%,

Table 2. Morphological characteristics of mango cultivars in Subang

Cultivar Name	Origin Collection	Morphology Character									
		LF	LL	LW	PL	LT	FLA	FLB	FLM	SUL	SDL
Apel	P	0	0	0	0	0	1	0	0	0	0
Arumanis	P, K, J	1	1	1	0	0	1	1	0	0	0
Beruk	P	1	1	1	1	0	1	1	0	0	0
Bapang	P, K, J	1	1	1	0	0	1	2	0	0	0
Cengkir	P, K	1	1	1	1	0	1	1	0	0	0
Cengkir Gajah	P	1	0	0	0	0	1	1	0	0	0
Cengkir Kebongan	P	1	1	0	0	0	1	2	0	0	0
GedongGincu	P	1	1	0	1	0	1	1	0	0	0
GedongKelapa	P	1	0	0	0	0	1	1	0	0	0
Gidang	P	1	1	0	0	0	1	1	0	0	0
Gincir	P	1	1	1	1	0	1	1	0	0	0
Golek	P	1	1	0	0	0	1	1	0	0	0
GolekNaga	P	1	1	0	1	0	1	1	0	0	0
Gori	P	1	1	1	0	0	1	2	0	0	0
Lenggak	P	1	1	0	0	0	1	1	0	0	0
MaduKeraton	P, K	1	1	1	1	0	1	1	0	0	0
Manalagi	P, K	1	1	1	1	0	1	1	0	0	0
Muncang	P	1	1	0	0	0	1	1	0	0	0
Nanas	P	1	1	1	0	0	1	1	0	0	0
Ompyong	P	1	1	1	1	0	1	1	0	0	0
TO	P	1	1	0	1	0	1	1	0	0	0

Note. P = Pamanukan; K = Subang; J =Cagak Street.

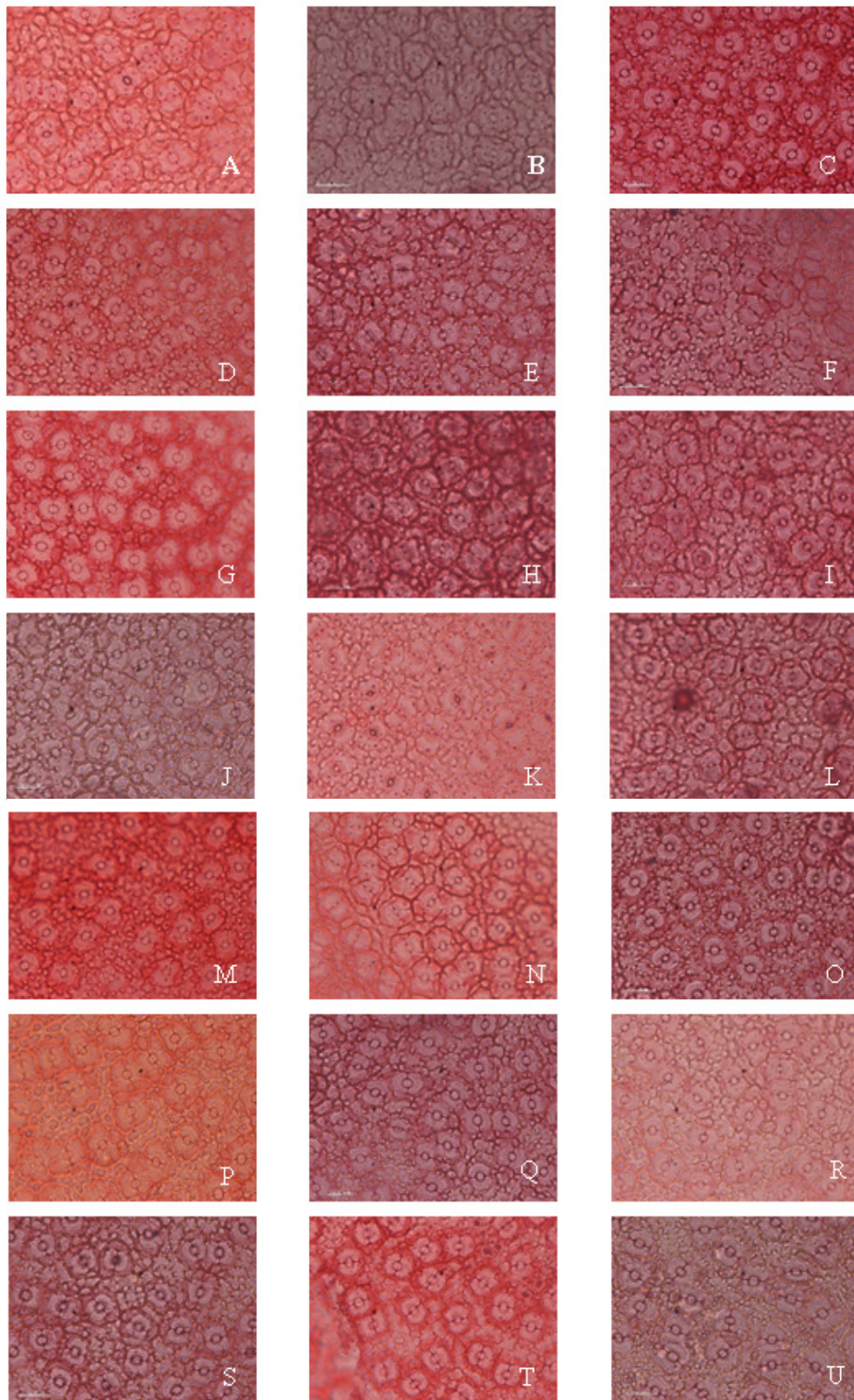


Figure 1. Paradermal abaxial section of leaves of mango cultivars (at 40x magnification). A. apel; B. bapang; C. beruk; D. cengkir; E. cengkir gajah; F. cengkir kebonan; G. gedong gincu; H. gedong kelapa; I. gidang; J. gincir; K. golek; L. golek naga; M. gori; N. arumanis; O. lenggak; P. madu keraton; Q. muncang; R. nanas; S. ompyong; T. manalagi; U. TO

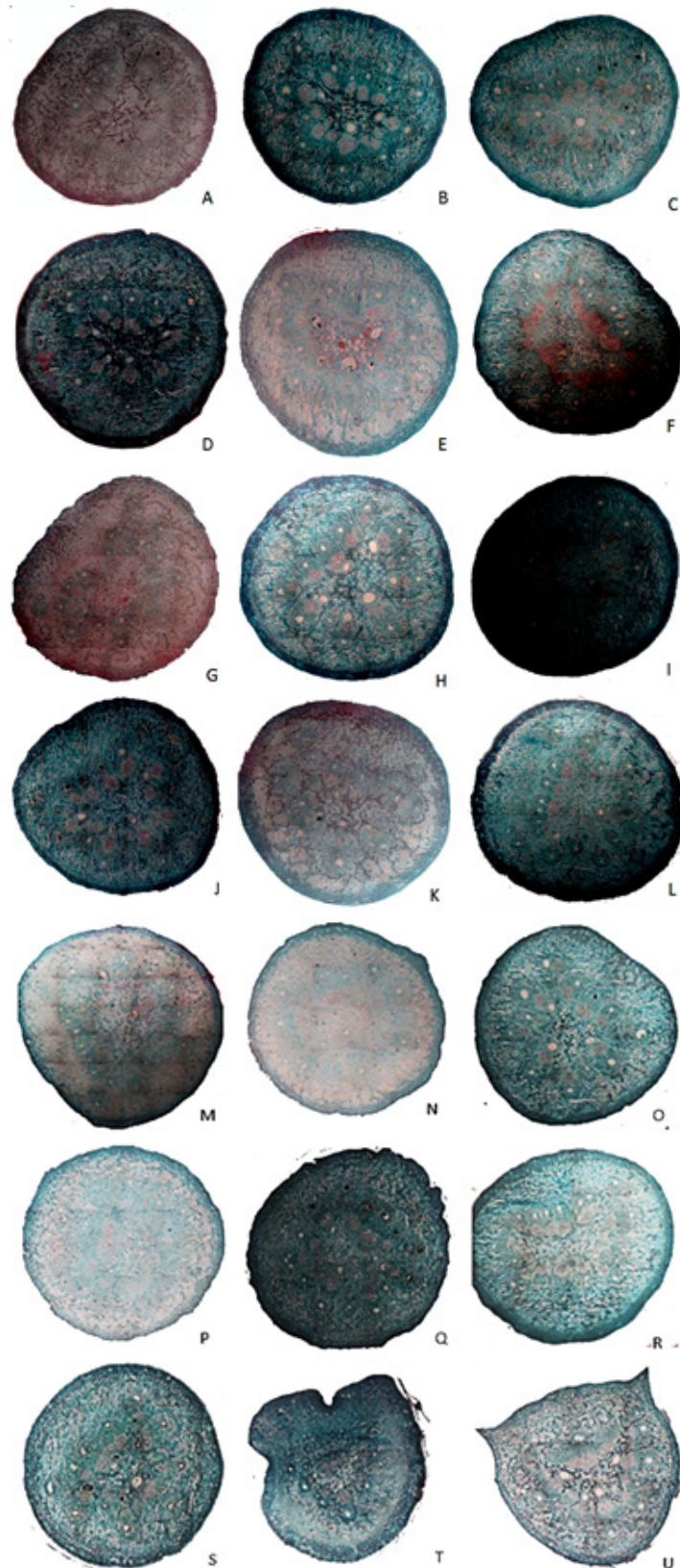


Figure 2. Cross section of leaf petiole (40x magnification). A. apel; B. Bapang; C. cengkir gajah; D. gincir; E. golek; F. cengkir kebongan; G. nanas; H. ompyong (*Planconvex*); I. gidang; J. beruk; K. cengkir; L. golek naga; M. gori; N. gedong gincu; O. Lenggak; P. madu kraton; Q. muncang; R. TO; S. manalagi (*Circular*); T. gedong kelapa; U. Arumanis (*Highlyconvex*)

Table 3. Characteristics of mango cultivars stomata in Subang

Cultivar Name	Collecting Site	Stomata Density (Σ stomata/mm ²)	Stomata Index (%)	Stomata Length (μ m)	Stomata Width(μ m)
Apel	Pamanukan	700.6	44.5	7.7 \pm 0.67	7.4 \pm 0.70
Arumanis	Pamanukan	668.0	45.2	8.0 \pm 0.66	7.4 \pm 0.51
	Subang	661.0	44.4	7.6 \pm 0.69	8.0 \pm 0.66
Beruk	Cagak Street	452.0	41.9	8.7 \pm 0.67	10.4 \pm 1.17
	Pamanukan	652.2	44.2	7.9 \pm 0.73	8.7 \pm 0.67
Bapang	Pamanukan	680.2	45.2	7.6 \pm 0.70	7.6 \pm 0.51
	Subang	641.4	44.6	7.9 \pm 0.73	8.0 \pm 0.67
	Cagak Street	456.0	41.9	9.7 \pm 1.33	10.7 \pm 1.15
Cengkir	Pamanukan	681.5	45.7	7.8 \pm 0.78	7.8 \pm 0.63
	Subang	602.5	44.7	8.0 \pm 0.66	8.2 \pm 0.63
Cengkir Gajah	Pamanukan	649.6	44.3	7.6 \pm 0.51	7.9 \pm 0.99
Cengkir Kebongan	Pamanukan	681.5	44.3	7.7 \pm 0.67	7.6 \pm 0.69
Gedong Gincu	Pamanukan	644.5	44.4	8.1 \pm 0.73	8.4 \pm 0.69
Gedong Kelapa	Pamanukan	685.3	45.2	7.7 \pm 0.67	7.9 \pm 0.87
Gidang	Pamanukan	669.0	43.7	7.7 \pm 0.67	8.1 \pm 0.87
Gincir	Pamanukan	687.0	43.2	7.6 \pm 0.69	7.8 \pm 0.63
Golek	Pamanukan	629.2	44.3	7.6 \pm 0.69	7.2 \pm 0.91
Golek Naga	Pamanukan	679.0	44.7	8.0 \pm 0.81	8.1 \pm 0.56
Gori	Pamanukan	670.0	45.5	7.8 \pm 0.63	7.7 \pm 0.67
Lenggak	Pamanukan	675.0	44.0	7.8 \pm 0.63	7.6 \pm 0.84
MaduKeraton	Pamanukan	669.0	46.0	8.4 \pm 0.84	7.8 \pm 0.78
	Subang	619.0	45.4	7.7 \pm 0.82	7.9 \pm 0.73
Manalagi	Pamanukan	657.3	45.0	7.8 \pm 0.78	7.2 \pm 0.91
	Subang	634.3	44.2	7.8 \pm 0.63	7.7 \pm 0.67
Muncang	Pamanukan	643.3	45.0	7.7 \pm 0.67	7.9 \pm 0.73
Nanas	Pamanukan	643.3	46.0	8.0 \pm 0.66	7.5 \pm 0.52
Ompyong	Pamanukan	698.0	44.0	8.0 \pm 0.66	8.0 \pm 0.94
T.O	Pamanukan	675.1	44.1	7.7 \pm 0.67	7.4 \pm 0.69

consisted of 7 cultivars: Beruk, Arumanis 2, Cengkir 1, MaduKeraton 2, Manalagi 1, Manalagi 2, Gori, Arumanis 1, Madu Keraton 1, Cengkir 2, Gedong Gincu, and Golek Naga. Among cultivars of the subgroup IIB, the highest similarity value (100%) recorded for Mango Cengkir 1, Madu Keraton 2, Manalagi 1 and Manalagi 2 while Gedong Gincu and Golek Naga belonged to another subgroup of subgroup IIB.

Group III had similarity value of 82% and consisted of Bapang 3, Arumanis 3 and Ompyong, which characterised by high stomatal density. Mango Bapang 3 (from Cagak Street) had larger stomatal size than Bapang 1 and Bapang 2. Mango Bapang 3 stomatal size separated it from

other Bapang cultivars. The similar case was also recorded for Arumanis of Cagak Street.

CONCLUSION

Intraspecific variation based on morphological leaf mango cultivars is considered in low category. Type of stomata on all mango cultivars was actinocytic and found only in abaxial parts (hypostomatic type). The inconsistency in density, size and stomatal index on the same cultivars which originated from different locations made those characters were unsuitable to be used as taxonomic characters. On the other hand, three types of carrier file forms (Plano-convex, highly

Table 4. Characteristics of the petiole anatomy mango cultivars

Cultivar Name	Form Carrier Network	Type Oxalate Crystal
Apel	Planoconvex	Drus small cubes
Arumanis	Highlyconvex,	Drus prismatic
Bapang	Planoconvex	Drus, prismatic
Beruk	Circular	Drus, prismatic
Cengkir	Circular	Drus, prismatic
CengkirGajah	Planoconvex	Drus
CengkirKebongan	Planoconvex	Drus, prismatic
GedongGincu	Circular	Drus, prismatic
GedongKelapa	Highly convex	Drus, small cubes
Gidang	Circular	Drus, prismatic
Gincir	Planoconvex	Drus, prismatic
Golek	Planoconvex	Drus, prismatic
GolekNaga	Circular	Drus, prismatic
Gori	Circular	Drus, prismatic
Lenggak	Circular	Drus
MaduKeraton	Circular	Drus, prismatic
Manalagi	Circular	Drus, prismatic
Muncang	Circular	Drus, prismatic
Nanas	Planoconvex	Drus, prismatic
Ompyong	Planoconvex	Drus
TO	Planoconvex	Drus, prismatic

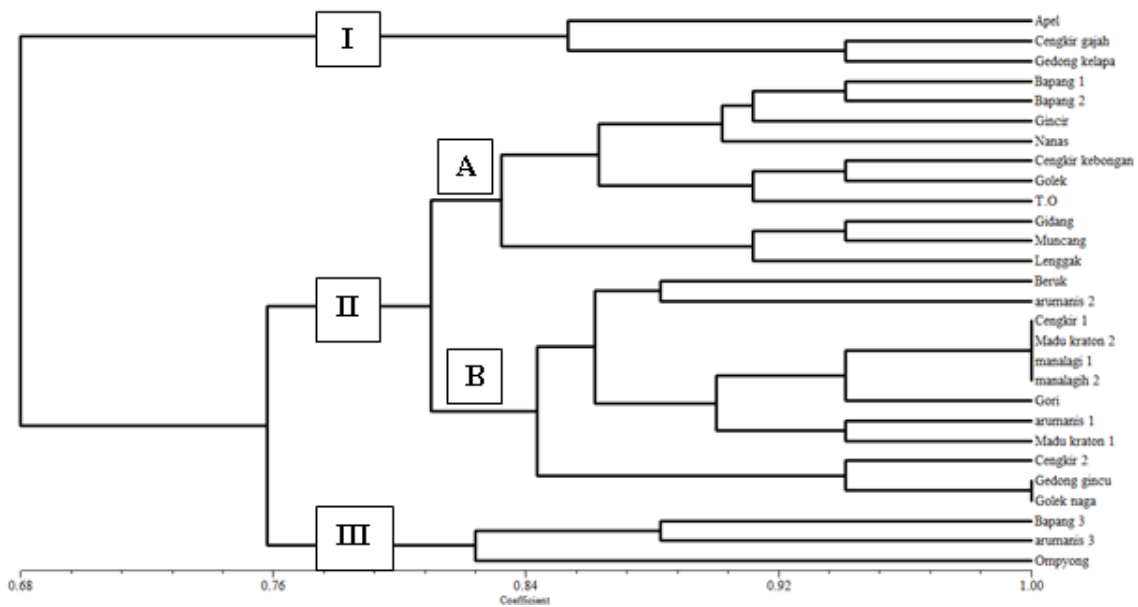


Figure 3. Dendrogram cultivars of mango based on the anatomy and morphology of leaves.

convex and circular) and availability of oxalate crystals in the leaf stalk can be used as characters for mango cultivar classification. Furthermore, more samples from other regions are necessary

to develop a specific taxonomic system for local mango cultivars.

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