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Morphological evaluation and determination keys of 21 citrus genotypes at seedling stage

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Abstract. *Budiarto R, Poerwanto R, Santosa E, Efendi D. 2021. Morphological evaluation and determination keys of 21 citrus genotypes at seedling stage. Biodiversitas 22: 1570-1579.* The identification of citrus varieties is generally based on flower, fruit, and mature tree characters. The detailed and comprehensive identification of seedling stage is very limited, therefore present study aimed to identify and distinguish 21 citrus genotypes based on 50 morphological characters of vegetative shoot at seedling stage. Cluster analysis using complete linkage agglomerative method showed broader dissimilarities between *C. x limon* and *C. x microcarpa*. Unfortunately, this method was limited to differentiate six genotypes within *Citrus reticulata* Blanco due to extremely low dissimilarities found. All citrus seedlings have similarities in the forms of habitus, gland spots, arrangement and venation of leaf. The result of PCA determined petiole wing, spine, color, hair and fragrance of leaves as five morphological markers at seedling stage. In addition, there was a positive correlation between spine and leaf pleasant. Moreover, the details of morphological dissimilarities between genotypes were described in arranged determination keys.

Keywords: *Citrus reticulata, Citrus x limon,* linkage agglomerative, morphology, vegetative shoot

INTRODUCTION

Citrus is economical important horticultural product worldwide (Spreen et al. 2020; Zhong and Nicolosi 2020), including Indonesia (FAO 2016). The use of good quality seedling could support the success of citrus agribusiness. High-quality citrus seedlings are ready to be released to the market five months after grafting and are characterized by high varietal purity or true to type, vigorous growth and being disease-free (Poerwanto and Susila 2014). Many farmers find difficulties accessing good quality seedlings when they start to grow, so they are forced to plant seedlings that lack quality checking, which may lead to them being disappointed during the harvest season (Setiono 2016).

Comprehensive seedling selection helps citrus farmers provide only good quality seedling for supporting the success of their agribusiness since quality checking allows them to select only either well growth seedling for transplanting or just to confirm desirable variety as their preference. Desirable variety of citrus could be recognized through morphological identification (de Oliveira et al. 2002; Koehler-Santos et al. 2003; Malik et al. 2012). This method is relatively simple, easy, cost- and time-saving (Dorji and Yapwattanaphun 2011). Although, the accuracy in terms of genetics is less than molecular techniques such as RFLP (Abkenar et al. 2004; Jena et al. 2009; Golein et al. 2012), AFLP (Xiao et al. 2009; Dorji and Yapwattanaphun 2015), and microsatellite (Ghanbari et al. 2009; Garcia-Lor et al. 2013; Rohini et al. 2020). The weaknesses of the molecular technique are relatively complicated, expensive and highly dependent on the sophistication of laboratory equipment (Ballve et al. 1997).

Varietal purity test is required to avoid confusion during citrus identification. It is required since the genus exhibit complex genetic relationships (Mabberley 2004). A high citrus diversity revealed at inter- and intra-specific levels is likely caused by wide sexual compatibility (Yu et al. 2017), leading to natural hybridization and spontaneous mutations (Dorji and Yapwattanaphun 2011). Citrus also spread over tropical and subtropical regions indicates the ability of this species to grow and adapt to various environmental conditions (Srivastava et al. 2000). There was 50 varieties among 250 citrus genotypes in Indonesia that have been released to the public (ICSFRI 2020).

Released citrus varieties are usually equipped with a description document. The document was dominated by morphological descriptions of flower, fruit, and tree characters. When the citrus had not yet shown either flower, fruit, or tree characters, the problem arose when the farmer would try to identify citrus varieties in seedling stage. This situation is observed in the description of RGL mandarin (*C. reticulata* Blanco) (Ibrahim 2012) and other *Citrus* species.

The only part that could be morphologically identified at seedling stage is vegetative shoot. The structure of vegetative shoots of seedlings composed of branches and leaves. Deeper morphological identification of the vegetative shoot is needed and important for plant breeders, seedling sellers or farmers. Previous study proved that leaf venation structure help to distinguish 4 common species of *Shorea* spp. with accuracy of more than 80% (Ariawan et al. 2020). Leaf shape is successful to solve some misunderstandings in the taxonomy of *Sagittaria trifolia* (Huang and Liu 2014). There was likely a relationship between taxonomic and morphology of plant, as Fitriana and Susandarini (2019) reported in shallot and Marboh et al. (2015) in citrus. Recently, the detailed and comprehensive identification of vegetative shoots of citrus genotypes at seedling stage is very limited. Therefore, the present study aimed to identify and distinguish citrus genotypes based on its vegetative shoot morphological characters at seedling stage.

MATERIALS AND METHODS

Study area

This study was conducted from November 2016 to March 2017. Various citrus genotypes were collected in November 2016 from two nurseries in two locations representing the distinct geographical conditions of Java island, Indonesia. The 1st nursery was Punten nursery that ICSFRI managed in the highland of Batu, East Java, Indonesia. The latitude, longitude and altitude of Punten nursery are -7.8416, 112.522624 and 954 m above sea levels (asl), respectively. The 2nd nursery was Agropromo nursery in low land of Bogor, West Java, Indonesia. The latitude, longitude and altitude of Agropromo nursery are -6.6000995, 106.8067535 and 266 m asl, respectively. All citrus seedlings were replanted in Pasir Kuda experimental farm of IPB in Bogor, Indonesia. The latitude, longitude, and altitude of the Pasir Kuda experimental garden are -6.609042, 106.783605, and 263 m asl.

Procedure

This study used 21 citrus genotypes that consisted of 13 different species at seedling stage. Some species such as *C. reticulata, C. nobilis, C. maxima, C. x sinensis, C. x limetta, C. x limonia* (C1 to C10) were collected from a nursery in Batu, while the rest of the species (C11 to C21) were obtained from Batu, Indonesia (Table 1). Each genotype consisted of four individuals with a uniform height of about 50 cm. The seedling was a five-months after grafting, except *C. x limonia* and *C. x jambhiri* that grow from seed or 8 months after germination.

The seedlings were re-planted into polybags filled with well-mixing soil and organic fertilizer (50:50). Seedlings were raised until the end of experiment (60 days after transplanting). Nitrogen-phosphorus-potassium compound fertilizer at a rate of 20 gram (Yara, Norway) and micronutrient (Growmore, USA) at a rate of 2 gram was applied monthly through soil drench and foliar feeding, respectively. Irrigation was not applied during the experiment due to the rainy periods experienced during the duration of the experiments.

Morphological observations were made on the dormant branch. A single vegetative shoot obtained from each individual seedling was used as an observation sample. Observed characters were only focussed on morphological variation of leaves arranged in a single fully developed branch. Observation forms were developed from Descriptors for Citrus (IPGRI 1999), Plant Morphology (Tjitrosoepomo 2009), Plant Terminology (Northern Ontario Plant Database 2017) and some morphological variation found during the running of experiment. The form consisted of 50 distinctive morphological characters (Table 2). Most of the observations were done by scoring, but some of the quantitative variables were measured by ruler.

Table 1. List of 21 citrus genotypes used in present studies and their common usefulness

Code	Scientific names	Intl. names	Local names	Usefulness
C1	*C. reticulata* Blanco	Mandarin	Keprok Rimau Gerga Lebong	Table fruit
C2	*C. reticulata* Blanco	Mandarin	Keprok Batu55	Table fruit
C3	*C. reticulata* Blanco	Mandarin	Keprok Terigas	Table fruit
C4	*C. reticulata* Blanco	Mandarin	Keprok JOP	Table fruit
C5	*C. nobilis*	Tangerine	Siam Pontianak	Table fruit
C6	*C. nobilis*	Tangerine	Siam madu	Table fruit
C7	*C. maxima* (Burm.) Merr.	Pummelo	Pamelo Magetan	Table fruit
C8	*C. x sinensis*	Oranges	Manis Pacitan	Juice
C9	*C. x limetta*	Sweet lime	Nipis manis	Juice
C10	*C. x limonia*	Rangpur lime	Japansche Citroen	Rootstock
C11	*C. x jambhiri*	Rough lemon	Rough lemon	Rootstock
C12	*C. x shiranui*	Dekopon	Dekopon	Table fruit
C13	*C.× aurantifolia* (Christm.) Swingle	Lime	Nipis lokal	Juice
C14	*C. x limon* (L.) Osbeck	Lemon	Lemon	Juice
C15	*C. amblycarpa* (Hassk.) Ochse	Nasnaran	Limau	Flavor
C16	*Citrus hystrix* DC.	Kaffir lime	Purut	Flavor
C17	*C. × microcarpa* Bunge	Calamondin	Kalamondin	Flavor
C18	*C. × microcarpa* Bunge	Variegated Calamondin	Kalamondin Varigata	Ornament
C19	*C. × microcarpa* Bunge	Calamondin	Kip	Ornament
C20	*C. japonica*	Kumquat	Marumi	Ornament
C21	*C. japonica*	Kumquat	Nagami	Ornament

Table 2. Vegetative shoot characters and their variation used for citrus seedling morphological identification

Characters	Variation
Habitus	Shrub, tree
Branch size	Small (>5mm), medium (6-10mm), Big (10-15mm),
Branch shape	Round, flat
Glandular spot on branch	Absence, presence
Hair on branch	Absence, presence
Spine	Absence, presence
Spine density	Spine is not grown in every node, spine grow in every node
Spine shape	Curved, straight
Spine length	Absence, small (>5mm), medium (6-10mm), large (10-15mm), very large (>15mm)
Gland spot on leaf	Absence, presence
Leaf arrangement	Alternate, opposite, whorled
Leaf lamina attachment*	Sessile, brevipetiolate, longipetiolate
Leaf division	Simple, unifoliate, trifoliate
Shoot tip surface	Glabrous, pubescent
Shoot tip color	Green, brown, yellow
Leaf-blade	Absence, presence
Petiole	Absence, presence
Stipule	Absence, presence
Variegation	Absence, presence
Young leaf color	Green, brown, yellow
Leaf-blade color comparison	Lighter upper than lower, similar, darker upper than lower
Adaxial color	Light green, green
Abaxial color	Yellowish green, light green
Lamina color compared to petiole	Different, similar
Leaf glossiness	Matt, glossy
Leaf surface	Uneven, even
Leaf flexibility	Rigid, flexible
Leaf thickness	Thin, fleshy
Leaf fragrance	Unpleasant, pleasant
Leaf venation	Pinnate, palmate, parallel, reticulate
Abaxial midrib	Flat, protuberant
Adaxial midrib	Flat, protuberant
Adaxial vein	Unclear, clear
Abaxial vein	Unclear, clear
Lamina length (cm)	Measured by ruler as quantitative data
Lamina width (cm)	Measured by ruler as quantitative data
Leaf phyllotaxy	3/1, 4/1
Leaf lamina shape*	Elliptic, ovate, obovate, lanceolate, orbicular, obcordate
Leaf apex*	Attenuate, acuminate, acute, obtuse, rounded, emarginate
Leaf margin*	Crenate, dentate, entire, sinute
Leaf base	Cuneate, obtuse, rounded, cordate
Leaf edge	Flat, wavy
Junction petiole and lamina*	Fused, articulated
Petiole wing	Absence, presence
Petiole wing shape*	Obcordate, obdeltate, obovate, linear
Petiole wing length	Very small (<=2mm), small (3-5mm), medium (6-10mm), large (10-15mm), very large (>15mm)
Petiole wing width	Very small (<=2mm), small (3-5mm), medium (6-10mm), large (10-15mm), very large (>15mm)
Petiole length	Short (3-5mm), medium (6-10mm), long (10-15mm), very long (>15mm)
Petiole shape	Round, flat
Petiole color	Yellowish green, green

Data analysis

The results were clustered by complete linkage agglomerative method with Gower coefficient using R stat 3.1.0 (http://www.r-project.org/). Principal component analysis (PCA) was also performed by similar statistical software. The determination key was prepared through manual selection of morphological characters that distinguished one to another. The construction of determination keys followed the pattern of clustering dendrogram.

RESULTS AND DISCUSSION

Morphological evaluation

This study revealed morphological similarities among 21 citrus genotypes in form of habitus, the presence of glands either on branches or leaves, the absence of leaf stipule, spirally alternate leaf arrangement; the darker adaxial (upper side) than abaxial (lower side); and pinnate venation with protuberant abaxial midrib. Those similarities were assumed to be distinguishing characters between the Citrus and other genera. Previous study

(Irsyam 2015) showed that Citrus genus had spiral leaf arrangement than Triphasia and Severinia.

This study used a principal component analysis (PCA) to show grouping pattern of 21 citrus genotypes based on seedling morphology (Figure 1). Four genotypes of Keprok (Rimau Gerga Lebong, Terigas, JOP and Borneo Prima) and two genotypes of Siam (Pontianak and Madu) seemed to have a high similarity, so that become a group. They shared a quadrant with a small group that consisted of two genotypes of *C. japonica* (Marumi and Nagami). Its morphological characters influenced the distribution of genotypes scattered in four quadrants of PCA. Among 50 characters, our results showed five notable morphological characters based on their vector value that can be used to distinguish citrus seedling, i.e., petiole wing, spine, color, hair and fragrance (Table 3).

In addition to PCA, present study also used clustering analysis to complete the information about grouping patterns based on seedling morphological data. Both dendrogram (Figure 2) and PCA confirmed a relatively similar grouping pattern, especially in cluster of *C. reticulata, C. x microcarpa,* and *C. japonica*. The cluster was broadly divided into two groups at dissimilarities coefficient by about 60%. The first group was citrus genotypes with petiole wing and the second ones with no petiole wing. The first group was consisted of *C. hystrix, C amblycarpa*, *C. x aurantifolia*, *C. x shiranui*, *C. x sinensis*, *C. maxima* and three genotypes of *C. x microcarpa*, while the rest of genotypes join in the second group. The first and second groups were further subdivided into several subgroups based on other characters that were fully described in the determination key.

Petiole wing

Petiole wing is the additional leaf blade attached to the petiole that varies in shapes and sizes depending on citrus genotypes (Figure 3). Petiole wing was assumed to serve as additional leaf blade with similar function. Leaf-blade is known as the site of photosynthesis and transpiration of citrus plant (Budiarto et al. 2019a) Based on the size of petiole wing, the order from the largest to the smallest genotypes was *C. hytrix* > *C. maxima* > *C. x shiranui* > *C. x sinensis* > *C. amblycarpa* = *C. aurantifolia* > *C. x microcarpa*. The biggest petiole wing found in *C. hystrix* was caused by having a longer petiole compared to main leaf blade, known as longipetiolate. The opposite is brevipetiolate, which is defined as any citrus genotypes with smaller petiole than the leaf blade, irrespective to the presence of wing on petiole (IPGRI 1999).

Based on the shape of petiole wing, various citrus genotypes were divided into two groups namely the obdeltate group consisted of *C. x microcarpa*, *C. x sinensis*, *C. x shiranui* and the obovate group consisted of *C. hystrix*, *C. amblycarpa*, *C. x aurantifolia*, *C. maxima*. None of the observed genotypes possessed obcordate types. Petiole wing appearances such as the inverse of egg-shape and deltoid shape with pointed end were called obovate and obdeltate, respectively (Harris and Harris 2006). Unfortunately, not all of citrus genotypes have petiole wing such as *C. x limon*, *C. x jambhiri*, *C. x limonia*, *C. x limetta*, *C. japonica* and *C. reticulata*. This may be caused by improper development of the petiole wing that is encoded by their genetic background. They only appear as slightly protrude lines on the existing surface of the petiole.

Hair

Leaf hair, also called trichome, is an additional part of the leaf similar to hair outgrowth of the epidermal tissue (Harris and Harris 2006). Trichome was used to increase plant resistance against biotic stress such herbivore and abiotic stress such as drought (Dalin et al. 2008). In the present study, leaf hair was found only in *C. maxima*. The size of hair was approximately ± 1 mm and could be sensed by touching. The hair was distributed evenly on branches and young shoot tips (Figure 4).

Color

There were four color characters observed in the present study, i.e., the color of shoot tip, young leaves, mature leaf blade, and petiole (Figure 5). The color of shoot tip and young leaves was mostly green, although certain *C. hystrix* and *C. x aurantifolia* showed brownish-green. The green color on adaxial side was generally darker than on the abaxial side. In dicotyledonous C_3 leaves, adaxial side was likely exposed to more sunlight (sun leaves) than the lower ones (shade leaves) (Terashima 1986; Terashima and Evans 1988). Most citrus varieties had green petiole, while certain genotypes such *C. x limonia*, *C. x limon*, and *C. japonica* Nagami showed yellowish green.

The presence of variegation in *C. x microcarpa* altered most of color characters. Variegated genotype was marked with spots of different colors (Harris and Harris 2006), i.e., yellowish-white. The presence of green color was minor both in leaves and branches. Variegated citrus genotypes experienced a lack of chlorophyll pigment (Hortmag 2009). Due to the lack of chlorophyll compared to normal ones, it is assumed that this type required a shady environment for optimal growth. Variegated characters in citrus seedlings were promoted by random cell mutation instead of environmental condition to be inherited to the offspring (Hortmag 2009).

Table 3. Selected morphological characters based on PCA of 21 citrus genotypes at seedling stage

Characters	**Sub characters**	**Vector value**	**PC group**
Petiole wing	The width of petiole wing	0.318	PC1
	The length of petiole wing	0.316	PC1
	The shape of petiole wing	0.311	PC1
	The presence of petiole wing	0.308	PC1
Hair	Hair on branch	0.124	PC1
	Hair on shoot tip	0.124	PC1
Color	Young leaf color	0.054	PC1
	The presence of variegation	0.055	PC1
Fragrance	Leaf fragrance	-0.007	PC1
Spine	The density of spine	-0.032	PC1
	The length of spine	-0.280	PC2
	The presence of spine	-0.271	PC2
	The shape of spine	-0.271	PC2

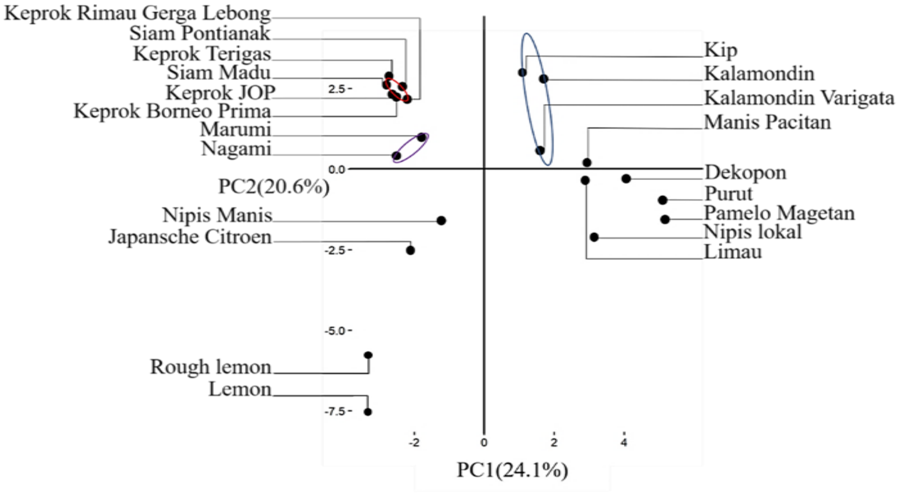

Figure 1. Principal component analysis (PCA) of morphological characters of 21 citrus genotypes at seedling stage

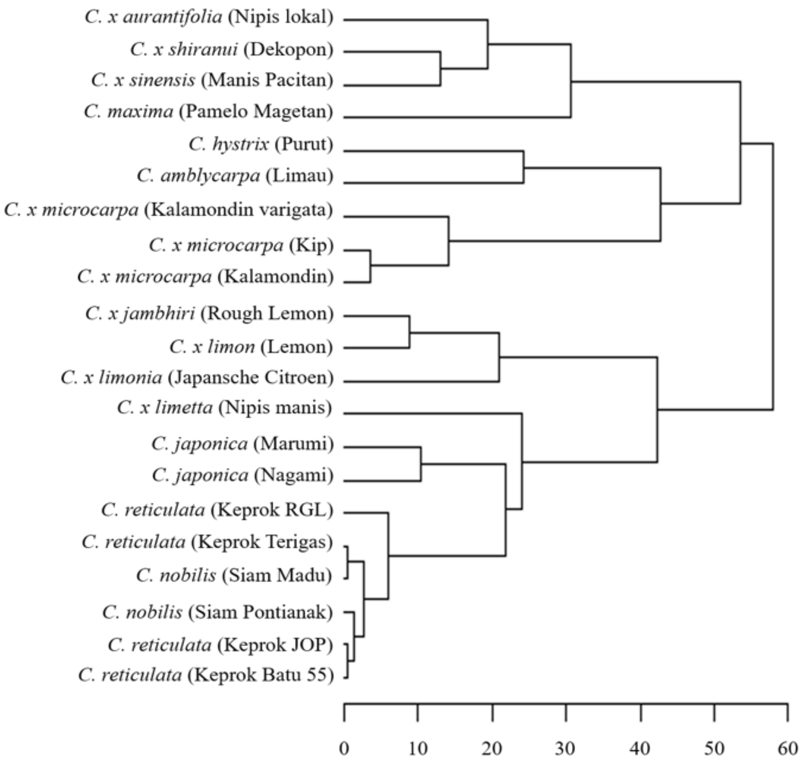

Figure 2. Dendrogram illustrating morphological dissimilarities of 21 citrus genotypes at seedling stage. Note: horizontal axes were coefficients of dissimilarities

Fragrance

Leaf fragrance can also be notable characters to identify citrus genotypes as some citrus genotypes released a strong and pleasant fragrance when the leaves were squeezed. The aromatic leaf of *C. hystrix* was the most famous among *Citrus* species (Figure 6.A). It was widely used to flavor dishes in most Asian nations, including Indonesia (Khoe and Mi 2015; Budiarto et al. 2019b). In addition to food spices, the pleasant fragrance leaves of *C. hystrix* could be used for essential oil production (Budiarto et al. 2019b; 2019b). Other genotypes such as *C. x limon*, and *C. x limonia* also had mild aroma, even though they were not as popular as *C. hystrix*. General knowledge of *C. x limonia* was used as rootstock (Khoe 2016), however present finding showed its potential as essential oil production due to fragranced leaves (Figure 6.B). Another fragrance species, namely *C. amblycarpa* was also reported to have potential as ornamental potted plant (Budiarto et al. 2017).

Spine

Spine is a modified leaf that resembled a sharp-pointed structure, fitted with vascular bundles, arisen from below the epidermis and located either in branch or stems of citrus plants (Harris and Harris 2006). Spine was a self-defense strategy of citrus against herbivores (War et al. 2012; Kariyat et al. 2017). The size and density of the spines varied among genotypes. Some citrus genotypes had small (<5 mm) and unevenly distributed spines, i.e., *C. x limetta*, *C. hystrix*, *C. x shiranui*, and *C. maxima*. Interestingly, the seedling of *C. x limonia* had the longest spine (10-15 mm) which was evenly distributed in every node of the branch (Figure 7). Citrus varieties that have large and evenly distributed spines on dense branching are usually used as protective hedges in landscape architecture (dos Santos et al. 2015). In this case, *C. x limonia*, also known as Japansche Citroen, is mostly used as rootstock due to its tolerance to abiotic and biotic stress (Singh et al. 2010, Poerwanto and Susila 2014; Khoe 2016; Yulianti et al. 2020).

For grower, the presence of the spine on citrus seedling is unfavorable (dos Santos et al. 2015) because it could increase production cost for handling adjustment during the packaging, transportation, transplanting, and field maintenance. Interestingly, the presence of spine positively correlated with the aromatic leaves, thus it can be used for early evaluation of the aromatic potentials of citrus genotypes at seedling stage in the future. Even though, not all citrus genotypes had spines, for example, *C. reticulata*, *C. japonica*, and *C. x microcarpa*. Since *C. x microcarpa* was offspring of *C. reticulata* and *C. japonica*, it showed similar traits such as the absence of spine (Mabberley 2004). Interestingly, the presence of spine positively correlated to the pleasant of leaf fragrance (Table 4).

Among 13 observed species, the highest dissimilarity was observed in between *C. x limon* and *C. x microcarpa*, whereas the smallest ones were found between *C. x limon* and *C. x jambhiri*. It is understood to be related to the genetic distance of the elders between those citrus species. The lower dissimilarities might be related to the closer genetic distance since *C. x limon* and *C. x jambhiri* shared the same elders, namely *C. medica* (Curk et al. 2016). In contrast, the elders of *C. x limon* were clearly different from *C. x microcarpa*. The elders of *C. x limon* were *C. medica* and *C. x aurantium* while *C. x microcarpa* was an offspring of *C. japonica* and *C. reticulata* (Mabberley 2004; Curk et al. 2016).

Morphological dissimilarities among 13 *Citrus* species (9-58%) were broader than those within a species (0.3-14%). This finding was in line with Syarif et al. (2017) that the morphological dissimilarities were found to be low within a species rather than among several species, because of the lower genetic variation within a species. In addition to genetic, morphological dissimilarities were also influenced by environmental conditions. Therefore, the present study replanted all citrus genotypes to the same field condition to eliminate the variation caused by environments.

Morphology based determination keys

The present study arranged determination keys among 13 *Citrus* species (Table 5) in order to help citrus identification at seedling stage. Thus, distinct characters described in the keys would be helpful for farmers to determine the desirable species for their agribusiness (Poerwanto and Susila 2014). For plant breeders, the determination key is used to do early selection in the offspring population at seedling stage (Ballve et al. 1997) and effectively manage citrus germplasm (Dorji and Yapwattanaphun 2011).

Additionally, present finding could also show relatively clear vegetative shoot variation within the same species of *C. japonica* (Table 6, Figure 8) and *C. x microcarpa* seedlings (Table 7, Figure 9). However, the limitation of present study emerged during determination of morphological difference within *C. reticulata* Blanco. The high morphological similarities observed among six genotypes of *C. reticulata* Blanco that composed of four Keprok varieties and two Siam varieties (Table 8; Figure 10). The name of Keprok is mostly replaced by mandarin in the international level of citrus trading. While previous work believed that Siam was a tangerine (Hassan et al. 2014) in tropical version, so it tended to greenish-yellow peel instead of orange ones (Sumiasih et al. 2017)

Both Keprok and Siam were reported to come from similar species, namely *C. reticulata*, since *C. nobilis* is confirmed as a synonym of *C. reticulata* (Irsyam 2015). The difference among six genotypes was only leaf size, however, leaf size character could be influenced by environmental factors instead of genetic ones, leading to the lack of proper morphological markers that linked to genetic background. Therefore, general practice to show detailed comparison within a Citrus species might still involve the fruit morphological characters, as Susandarini et al. (2013) did in *C. maxima* and Taylor (2017) in *C. reticulata*.

Table 4. Spearman correlation coefficient of five morphological characters of 21 citrus genotypes at seedling stage

Characters	Hair	Spine	Color	Fragrance
Hair				
Spine	0.23			
Color	-0.05	-0.21		
Fragrance	-0.13	0.59	-0.13	
Petiole wing	0.26	0.33	0.26	-0.03

Note: The coefficient correlation in grey cell is significant, p-value <0.05

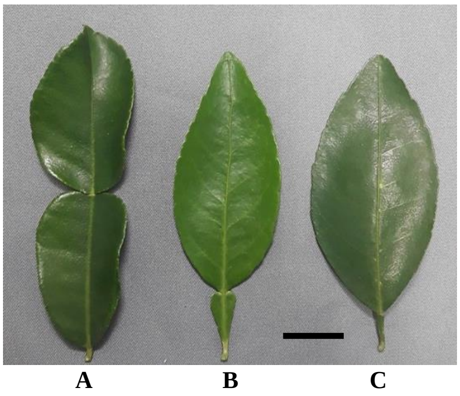

Figure 3. Petiole wing variation in citrus seedlings such as very big and obovate petiole wing supported by longer petiole in *Citrus hystrix* (A); small and obdeltate petiole wing in *C. x shiranui* (B); the absence of petiole wing in *C. reticulata* (C). Bar = 2 cm

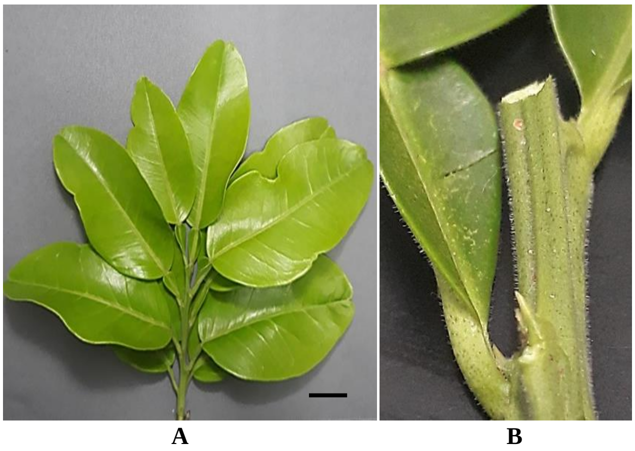

Figure 4. *Citrus maxima* (A) and its hairs in both branch and leaf (B). Bar = 2 cm

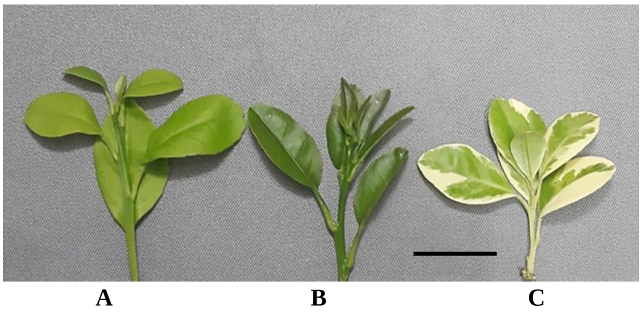

Figure 5. Color variation in young vegetative shoot of citrus seedlings; light green in *Citrus reticulata* (A); brownish-green in *C. x aurantifolia* (B); yellowish-white in variegated *C. x microcarpa* (C). Bar = 2 cm

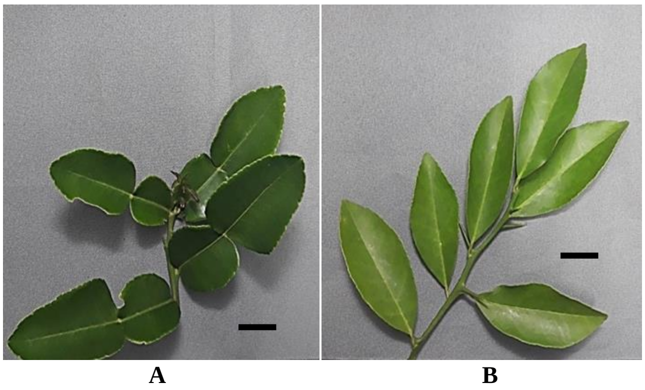

Figure 6. Two citrus genotypes with aromatic leaves; *Citrus hystrix* (A) and *C. x limonia* (B). Bar = 2 cm

Figure 7. Spines variation in citrus seedlings such as the absence of spines in *Citrus reticulata* (A); small straight spines in every node of *C. x aurantifolia* (B); very long and sharp-pointed spines in every node of *C. x limonia* (C). Bar = 2 cm

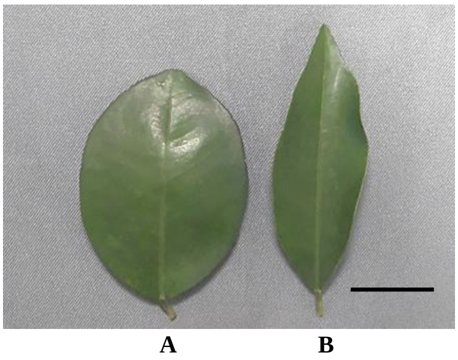

Figure 8. Leaf shape variation of two *Citrus japonica* genotypes, i.e Marumi (A) and Nagami (B) at seedling stage. Bar = 2 cm

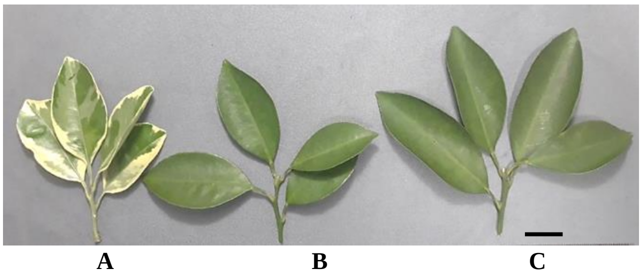

Figure 9. Vegetative shoot variation of three *Citrus x microcarpa* genotypes, i.e. Kalamondin Varigata (A), Kip (B) and Kalamondin (C) at seedling stage. Bar = 2 cm

Table 5. Morphology based determination key of 13 *Citrus* species at seedling stage

Code	Variation (continued to next code or end to species name)
1a	Petiole without wing (2)
1b	Petiole with wing (7)
2a	Spine in every node, pleasant fragrance (3)
2b	Spine in certain node or absence, unpleasant fragrance (5)
3a	Big spine, green young leaf, long petiole (*C. x limonia*)
3b	Medium spine, brownish-green young leaf, short petiole (4)
4a	dentate margin and ovate leaf (*C. x limon*)
4b	crenate margin and elliptic leaf (*C. x jambhiri*)
5a	Small spine, bold adaxial vein (*C. x limetta*)
5b	Absence spine, unbold adaxial vein (6)
6a	Fleshy leaf, entire margin, flat edge (*C. japonica*)
6b	Thin leaf, crenate margin, wavy edge (*C. reticulata*)
7a	Bold abaxial vein (8)
7b	Unbold abaxial vein (11)
8a	Big and hairy branch, bold adaxial vein, entire leaf margin (*C. maxima*)
8b	Medium and no hair branch, unbold adaxial vein, crenate leaf margin (9)
9a	Round branch, spine in every node, obovate wing (*C. x aurantifolia*)
9b	Flat branch, spine in certain node, obdeltate wing (10)
10a	4/1 phyllotaxy, rounded base, flat edge (*C. x shiranui*)
10b	3/1 phyllotaxy, obtuse base, wavy edge (*C. x sinensis*)
11a	Pleasant fragrance, with spine (12)
11b	Unpleasant fragrance, without spine (*C. x microcarpa*)
12a	Brevipetiolate, green tip and young leaves (*C. amblycarpa*)
12b	Longipetiolate, brown tip and young leaves (*C. hystrix*)

Table 6. Morphology based determination key of two *Citrus japonica* genotypes at seedling stage

Code	Variation (genotypes name)
1a	Lanceolate lamina, long and yellowish-green petiole (Nagami) (Figure 8.B)
1b	Orbicular lamina, medium and green petiole (Marumi) (Figure 8.A)

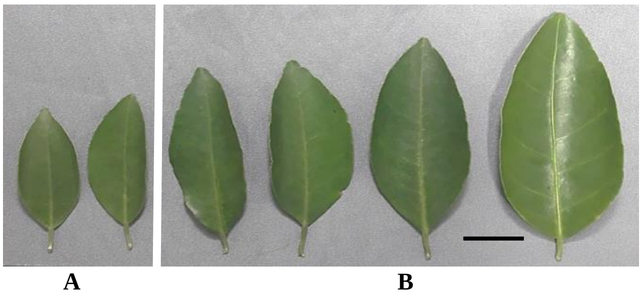

Figure 10. Leaf size variation of six *Citrus reticulata* genotypes; Keprok Trigas, Siam Madu (A); Keprok JOP, Keprok Batu 55, Siam Pontianak and Keprok RGL (B) at seedling stage. Bar = 2 cm

Table 7. Morphology based determination key of three *Citrus x microcarpa* genotypes at seedlings stage

Code	Variation (continued to next code or end to species name)
1a	Variegation, yellow tip (Kalamondin varigata) (Figure 9.A)
1b	No variegation, green tip (2)
2a	Obtuse leaf base, long petiole (Kalamondin) (Figure 9.C)
2b	Cuneate leaf base, medium petiole (Kip) (Figure 9.B)

Table 8. Morphology based determination key of six *Citrus reticulata* genotypes at seedling stage

Code	Variation (genotypes name)
1a	Small leaf, width < 2.5 cm, length < 6 cm, both ratio 2.4-2.6 (Siam Madu, Keprok Trigas) (Figure 10.A)
1b	Big leaf, width > 2.5 cm, length > 6 cm ratio, both ratio 2.1-2.2 (Siam Pontianak, Keprok Batu 55, Keprok JOP, Keprok Rimau Gerga Lebong/RGL) (Figure 10.B)

In conclusion, both clustering analysis and PCA confirmed a relatively similar grouping pattern, especially in cluster of *C. reticulata*, *C. x microcarpa*, and *C. japonica*. The highest dissimilarity was observed in between *C. x limon* and *C. x microcarpa*. Unfortunately, this method was limited to differentiate six genotypes within *C. reticulata* due to extremely high similarities found. PCA results showed five notable characters to distinguish citrus seedling such as petiole wing, hair, color, fragrance, and spine. Moreover, the presence of spine was significant and positively correlated to the pleasant leaf fragrance. The determination keys were constructed to ease the morphological evaluation of 21 citrus genotypes at seedling stage.

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