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 leaves. 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 (Spree 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 266 m 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 -6.600995, 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. × sinensis*, *C. × limetta*, *C. × 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. × limonia* and *C. × 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.

| Code | Scientific names | Intl. names | Local names | Usefulness |
|------|------------------|-------------|-------------|------------|
| C1   | *C. reticulata*  | Mandarin    | Keprok Rimau Gerga Lebong | Table fruit |
| C2   | *C. reticulata*  | Mandarin    | Keprok Batu55 | Table fruit |
| C3   | *C. reticulata*  | Mandarin    | Keprok Terigas | Table fruit |
| C4   | *C. reticulata*  | 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. | Pummmelo | Pamela Magetan | Table fruit |
| C8   | *C. × sinensis*  | Oranges     | Manis Pacitan | Juice |
| C9   | *C. × limetta*   | Sweet lime  | Nipis manis | Juice |
| C10  | *C. × limonia*   | Rangpur lime| Japansche Citroen | Rootstock |
| C11  | *C. × jambhiri*  | Rough lemon | Rough lemon | Rootstock |
| C12  | *C. × shiranui*  | Dekopon     | Dekopon | Table fruit |
| C13  | *C. × aurantifolia* (Christm.) Swingle | Lime | Nipis lokal | Juice |
| C14  | *C. × 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, brevpetiolate, longpetiolate                                     |
| Leaf division                  | Simple, unifoliate, trifoliolate                                          |
| 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, denteate, 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
(Irsham 2015) showed that Citrus genus had spiral leaf arrangement than Triphasis 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 (Ponkritam 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 (Maruma 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 shiraniu, 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. hystrix > C. maxima > C. x shiraniu > 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 longipetiolute. The opposite is brevipetiolute, 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 shiraniu 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 (Dalim 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. lebong, 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      |
| Fragrance       | The presence of variegation | 0.055     | PC1      |
| Spine           | Leaf fragrance       | -0.007       | PC1      |
|                 | 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 shiruanu, 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; 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 (Cukr 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; Cukr 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 (Suniasih 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      | 0.23 | -     | -     | -         |
| Spine     | -    | 0.50  | -0.13 | -0.03     |
| Color     | -0.05| -0.21 |       |           |
| Fragrance | -0.13| 0.50  | -0.03 |           |
| 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.

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 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) |

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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