

## Assessment of Taxonomic Affinity of Indonesian Pummelo (*Citrus maxima* (Burm.) Merr.) Based on Morphological Characters

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### ABSTRACT

*Citrus maxima* is a tropical fruit species with high morphological variability on fruit characters, including shape, size, thickness of peel, flesh color and taste. This variability has been used by breeders and common people to practically distinguish different pummelo cultivars. Despite such well known variation, so far there is no published study specifically addressed the extent of phenotypic variability and taxonomy of pummelo in Indonesia. This study was, therefore, aiming at assessing phenotypic variability and taxonomic affinity of Indonesian pummelo cultivars and landraces using multivariate analysis methods. A total of 60 accessions from six provinces were collected, including some representatives of registered cultivars. Twenty one morphological characters were subjected to cluster analysis and principal component analysis. Results showed that two major clusters were formed, in which each group was characterized by combination of distinctive morphological features leading to recognition of two cultivar groups. Based on the fact that the morphology of individuals from intensively cultivated orchards showed similar level of variability to those from sporadically distributed areas, there was strong indication that the morphological variation has genetic background.

**Keywords:** *Citrus maxima*, Taxonomy, Morphology, Phenetic Approach

### 1. INTRODUCTION

Pummelo (*C. maxima*, or formerly known as *C. grandis*) is a tropical fruit plant species originated from South East Asia, which in the western world is called as shaddock (Uzun and Yesiloglu, 2012). This species usually produces fruits twice a year, grows on various soil types at the altitude of 100-400 m above sea level (Dinesh and Reddy, 2012). From taxonomical point of view, *C. maxima* is considered to be one of three true *Citrus* species together with *C. medica* and *C. reticulata* based on karyotype analysis (Hynniewta *et al.*, 2011). Its status as true or basic species within *Citrus* is confirmed by other

researchers based on molecular analysis (Froelicher *et al.*, 2011; Garcia-Lor *et al.*, 2013).

*C. maxima* is considered as an easily recognized species due to a number of notable morphological characteristics, such as huge leaves borne on broadly winged petioles, very large and fragrance flowers and big fruits with a single embryo, while most of other *Citrus* species are polyembryonic (Uzun and Yesiloglu, 2012). The high phenotypic variability of Indonesian pummelo resulted in the existence of various names applied to the same cultivars. The problem is even being more complicated when different names are applied to commercial cultivars from different regions of the

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country. This problem may in turn lead to a more serious issue in determining the strategy for conservation due to uncertainty on its true biological diversity.

Pummelo as a plant species with long history of cultivation is facing a risk of decreasing variability as a result of artificial selection. In this regard, Pickersgill (2009) pointed out that cultivated plants experiencing artificial selection during domestication process may face a problem of declining variability through elimination of unwanted phenotypic characters that might result in decreasing genotypic variation. Wen *et al.* (2010) noted an urgent need for pummelo conservation as there is a tendency of declining varietal diversity due to loss of natural habitat. Therefore, the problem of decreasing variability in pummelo needs a particular attention in preventing further loss of plant species that has not been fully uncovered.

Morphological properties have been the main character used for recognition and description of plant taxa (Duminil and Michele, 2009; Dwari and Mondal, 2011). The important role of morphology is attributable to its advantageous properties such as ease of examination, has high variation, has an established descriptive terminology and its accessibility to herbarium specimens (Szczepaniak and Ciésłak, 2011). Moreover, morphology is applicable to all levels in taxonomic hierarchy. Morphology has always been the first taxonomic evidence for the recognition and delimitation of infraspecific categories. The key role of morphology in defining infraspecific taxa is shown by the fact that no formal taxonomic status would be assigned when there is no morphological differences among populations of particular plant species (Cires *et al.*, 2009; Lohwasser *et al.*, 2010).

The objective of this study is to assess phenotypic variability and taxonomic affinity of Indonesian pummelo accessions based on phenetic analysis on morphological characters. The implication on the species variability to its conservation is addressed accordingly.

## 2. MATERIALS AND METHODS

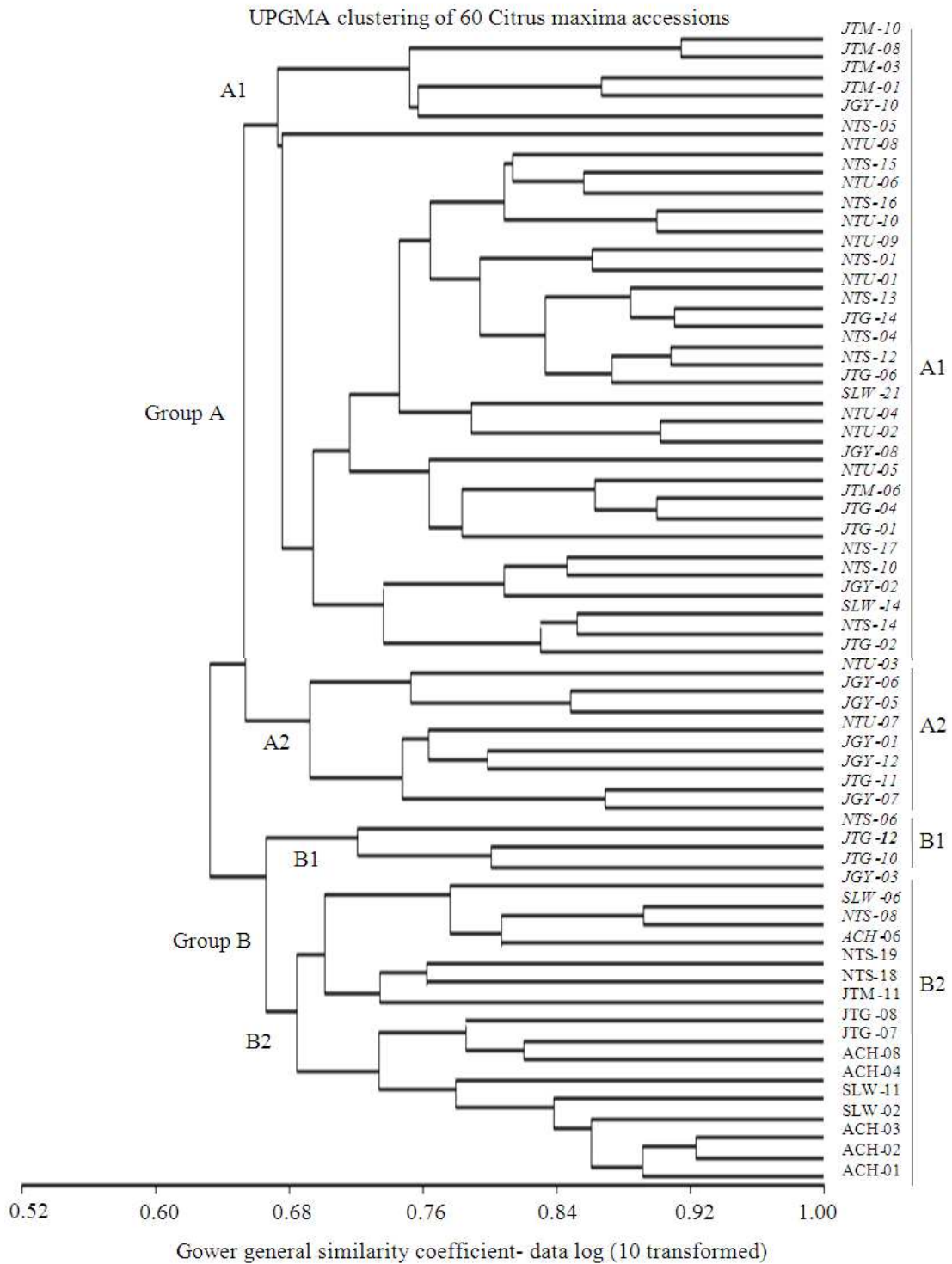
Plant materials were collected from six provinces in Indonesia, covering representatives of pummelo commercial cultivars and landraces. The commercial cultivars were obtained from two pummelo orchard areas (East Java and South Sulawesi), whereas the landraces were collected from four provinces representing species' sporadic distribution areas (Yogyakarta, Central Java, Aceh and East Nusa Tenggara). Morphological characters were examined by referring to standardized descriptor produced by International Plant Genetic

Resources Institute. Cluster analysis was performed to examine the grouping of accessions and to assess taxonomic affinity, while Principal Component Analysis (PCA) was done to evaluate the relative contribution of each character in the grouping of accessions. Gower's general similarity coefficient was calculated as a measure of similarity among accessions. The Unweighted Pair-Group Method Using Arithmetic Average (UPGMA) clustering technique was used to construct dendrogram illustrating taxonomic affinity of accessions. Data analysis was performed using Multivariate Statistical Program (MVSP) version 3.1 (Kovach Computing Services, UK).

## 3. RESULTS

A total of 60 accessions were collected, including 17 accessions representing 11 commercial cultivars and 43 accessions representing landraces. Observation on leaves morphology revealed a number of variability among accessions, including shape of leaf blade, leaf base, leaf apex, leaf margin, length of petiole, shape of petiole wing, ratio of leaf blade to petiole wing and the nature of connection between leaf blade and petiole wing. Flowers morphology varied only on their size and relative position of stamens to pistil. Fruits showed variability in their shape, apex, base, peel texture, appearance of oil glands on the peel, mesocarp color, number and arrangement of fruit segments, fruit flesh color and texture and number of seeds. Since flowers were not always available in all samples, thus the data used for subsequent analysis was consisted of morphological characters from leaves and fruits. Twenty one morphological characters, consisted of 15 qualitative and 6 quantitative, as shown in **Table 1** were then subjected to cluster analysis and principal component analysis.

Result of cluster analysis on 60 accessions of pummelo based on 21 morphological characters was shown in **Fig. 1**, with the grouping of accessions was developed based on Gower's general similarity coefficient. This coefficient is suitable for the data with mixed of qualitative and quantitative attributes (Solaro, 2010). The dendrogram constructed using UPGMA method showed that two main clusters were formed. The first cluster (labeled as group A) consisted of pummelo accessions commonly known as "Sour and Bitter Pummelo", whereas the second cluster (group B) was comprised of accessions known as "Sweet Pummelo". Each group could be recognized by combination of characters consistently clustered accessions belong to the corresponding group and thus combination of these characters was regarded as distinguishing characters for the group.



**Fig. 1.** Dendrogram showing the grouping of 60 pummelo accessions based on morphological characters

**Table 1.** List of morphological characters

Code	Characters	Character states
L01	Shape of leaf blade	(1) elips; (2) ovate; (3) obovate; (4) orbicular
L02	Leaf base	(1) acute; (2) acuminate
L03	Leaf apex	(1) acute; (2) rounded
L04	Leaf margin	(1) crenate; (2) entire
L05	Leaf length to width ratio	< 1,5; (2) 1,5 – 1,9; (3) > 1,9
L06	Petiole length	(1) short: <0,5 cm; (2) medium: 0,5-0,8 cm; (3) long: >0,8 cm
L07	Shape of petiole wing	(1) obcordate; (2) obdeltate; (3) obovate; (4) linear
L08	The nature of connection between leaf blade and petiole wing	(1) imbricate; (2) valvate; (3) separate
L09	Leaf length to petiole wing length ratio	< 3,5; (2) 3,6 – 5,0; (3) > 5,0
L10	Petiole wing length to width ratio	< 1,0; (2) 1,0 – 1,5; (3) > 1,5
F01	Fruit shape	(1) spherical; (2) elliptical; (3) pyriform (pear-shape); (4) obloid
F02	Fruit apex	(1) rounded; (2) flat; (3) concave
F03	Fruit base	(1) rounded; (2) flat; (3) concave
F04	Fruit peel texture	(1) smooth; (2) medium rough; (3) rough
F05	Oil glands on fruit peel	(1) inconspicuous; (2) conspicuous; (3) strongly conspicuous
F06	Mesocarp color	(1) white; (2) yellowish white; (3) pink; (4) red
F07	Number of fruit segments	10 – 14; (2) 15 - 18
F08	Uniformity and arrangement of fruit segments	(1) not uniform and irregularly arranged and ; (2) uniform and regularly arranged
F09	Fruit flesh color	(1) white; (2) yellowish white; (3) pink; (4) red
F10	Fruit flesh texture	(1) soft; (2) medium soft; (3) firm 21. F11 Number of seeds in fruit (1) seedless; (2) few: 5-10; (3) medium: 11-20; (4) numerous: >20

Group A was characterized by a wide, obcordate or obdeltate petiole wing, imbricate or valvate nature of leaf blade and petiole wing, leaf blade with crenate margin, medium rough to rough peel and the existence of numerous seeds in fruit. Meanwhile, the group B was characterized by a narrow, obovate or linear petiole wing, leaf blade and petiole wing were not connected to each other, leaf blade with entire margin, smooth or medium rough peel and few or medium number of seeds in fruit. There were some accessions with seedless fruits. Morphological characteristics of of these two groups were summarized in **Table 2**.

Group A could be divided further into two subgroups, assigned as A1 and A2 in the dendrogram. Morphological characters differentiating these two subgroups were fruit flesh color, texture of fruit flesh, number of seeds, peel texture, appearance of oil glands on the rind and association between leaf blade and petiole wing. Most of the accessions belong to the group A (Sour and Bitter Pummelo) are considered to be less preferred varieties, except those of subgroup A1. This less preferred varieties has sour fruit taste with various degrees of bitterness. They were the accessions representing landraces from various localities that usually grown as home yard plants.

Within the group B, there were three accessions (JGY-12, JTG-11 and JGY-07) with intermediate characters, a mixture between characteristics of group A

and B. The intermediate nature of these three accessions, those of subgroup B1, was clearly shown by their position in the dendrogram. The rest of accessions in the group B (labeled as subgroup B2 in the dendrogram) could be divided further into two sub clusters based on fruit flesh color. Nine accessions have pink or red flesh, while the other six were those with white or yellow flesh. Most of the accessions in these two sub clusters were consisted of widely known commercial cultivars.

Results of PCA showed that first principal component, which corresponded to the first axis, represented 16.42% of total variance, whereas the second principal component which was represented 29.19% of variance. The eigen value of each morphological character in the first and second axis of PCA was listed in **Table 3**, indicated the contribution of characters in the group formation. The first principal component was strongly correlated to three characters separating group A and B on the dendrogram, as indicated by the eigen value of higher than 0.3. These three characters were shape of petiole wing, the nature of connection between leaf blade and petiole wing and length to width ratio of petiole wing (L07, L08 and L10). Meanwhile, three most prominent characters in the second axis were fruit apex, fruit peel texture and number of seeds in fruit (F02, F04 and F11). Combination of these six characters was considered as having considerable contribution on the grouping of accessions.

**Table 2.** Morphological characteristics of two pummelo groups

Morphological character	Group A: Sour and Bitter Pummelo	Group B: Sweet Pummelo
Shape of petiole wing	Obcordate or obdeltate	Obovate or linear
Leaf length to petiole wing length ratio	Big (wide petiole wing)	Small (narrow petiole wing)
The nature of connection between leaf blade and petiole wing	Imbricate or valvate	Separate
Leaf margin	Crenate	Entire
Rind texture	Medium rough or rough	Smooth or medium rough
Number of seeds in fruit	Numerous	Few or medium

**Table 3.** Eigen value of morphological characters

Character code	Character	Eigen value	
		1st axis	2nd axis
L01	Shape of leaf blade	-0.019	0.172
L02	Leaf base	-0.292	-0.160
L03	Leaf apex	0.249	0.059
L04	Leaf margin	0.177	-0.203
L05	Leaf length to width ratio	0.160	-0.036
L06	Petiole length	-0.074	0.007
L07	Shape of petiole wing	0.417	0.288
L08	The nature of connection between leaf blade and petiole wing	0.463	0.215
L09	Leaf length to petiole wing length ratio	0.289	0.121
L10	Petiole wing length to width ratio	0.321	-0.083
F01	Fruit shape	-0.038	0.281
F02	Fruit apex	-0.064	0.324
F03	Fruit base	-0.057	0.261
F04	Fruit peel texture	-0.060	0.303
F05	Oil glands on fruit peel	-0.119	0.125
F06	Mesocarp color	-0.191	-0.002
F07	Number of fruit segments	-0.207	0.029
F08	Uniformity and arrangement of fruit segments	0.068	0.141
F09	Fruit flesh color	-0.146	-0.046
F10	Fruit flesh texture	-0.119	0.240

Based on the fact that each group formed on cluster analysis could be clearly distinguished by combination of characters consistently shared by members of the group, then the grouping of accessions resulted from phenetic analysis provided a reasonable basis for the recognition of cultivar groups for practical classification of Indonesian pummelo. Combination of two phenetic methods employed in this study, cluster analysis and principal component analysis, was used to provide strong empirical support on the role of morphology in the grouping of accessions and assessing their taxonomic affinity.

#### 4. DISCUSSION

The grouping of accessions resulted from cluster analysis which represented considerable degree of phenotypic variability did not show any correlation

pattern to geographical origin of the samples. Members of a particular cluster were consisted of accessions from various sampling areas and thus indicated that phenotypic variability was not influenced by their habitat or other environmental factors. Similar result was reported by Putra *et al.* (2010) on *Musa* sp. cv. Rastali from Peninsular Malaysia in which morphological variability was not correlated with geographical distribution. Discordant pattern between morphological and geographical grouping was also found on durum wheat (Aghaee *et al.*, 2010). Moreover, Brus *et al.* (2011) on their study on morphological variation of *Juniperus oxycedrus* using 12 characters from leaves and fruit noted that morphological variation among populations did not show any geographical differentiation. Results of these studies indicated that geographical separation did not play as a



driving force for phenotypic variability. Another implication drawn from phenetic analysis of pummelo was that morphological characters used in this study represented stable taxonomical characters in terms that their variability was not affected by environmental factors. Based on the fact that the widely known commercial cultivars from different localities retained their agromorphological characteristics, it was reasonable to affirm that this phenotypic variability had a genetic basis.

Result of the principal component analysis showed that among 21 morphological characters used in this study, 6 of them were categorized as having high diagnostic function in the differentiation of accessions into two major groups as designated by cluster analysis. Their contribution was indicated by their eigen value of higher than 0.3 on both axis 1 and axis 2 of the PCA result (**Table 3**). The use of eigen value  $>0.3$  as a criteria for marking characters with high diagnostic function referred to Sanni *et al.* (2010). These 6 characters comprised of 3 from leaves (L07, L08 and L10) and 3 from fruit (F02, F04 and F11). Considering the role of multivariate analysis in plant systematics studies, two most common methods used were the cluster analysis and principal component analysis (Makinde and Ariyo, 2010). These two methods functions at recognizing patterns of grouping, in which both of them do not based on a priori assumption so that have an advantage feature in the objectivity of the resulted grouping.

The role of leaves morphological characters as diagnostic feature for delimitation of infraspecific taxa has been reported by Mulumba and Kakudidi (2011) in *Acacia senegal*. Similar results was reported by Moore *et al.* (2010) on *Smilax*, in which identification of specimens based on leaves morphological characters was proven to be an effective approach since it could be applied for plants in their vegetative growth phase without having to wait until the flowering or fruiting period. Meanwhile, Viscosi and Cardini (2011) noted the importance of leaf morphology in taxonomy and thus developed a protocol to facilitate the use of leaf characters in numerical taxonomy. Moreover, Lu *et al.* (2012) reported that analysis on the leaf architectural types was useful for the identification and classification of *Camelia* and thus gave more support on the role of leaves morphology in taxonomy.

Result of principal component analysis indicated that fruit morphology contributed a considerable role to the differentiation of pummelo accessions into their corresponding groups. The role of fruit morphology in

plant taxonomy has been documented by Cadena-Iniguez *et al.* (2008) successfully identified eight biotypes on *Sechium edule* based on fruit characters, especially fruit shape, fruit color and features of fruit surface. Moreover, these characters were used to predict the evolutionary pathway of the species. Meanwhile, Oberle and Esselman (2011) based on their study on *Dodecatheon* found that fruit and seeds provided diagnostic morphological characters for distinguishing *D.frenchii* from *D.meadia*.

Results of this study also suggested that leaves and fruit morphological characters provided a practical approach in distinguishing cultivar groups within pummelo and thus applicable for common people and breeders in selecting favorable cultivars. This finding was indicated by the fact that group B, the Sweet Pummelo group, was consisted of favorable accessions mostly found in production centers of pummelo in Central Java, East Java and South Sulawesi. The practical use of morphological characters for the recognition of cultivars in horticultural plant species has been reported by several authors. In this regard, Asudi *et al.* (2010) used combination of leaves and fruit characters to classify *Carica papaya* accessions. In addition, Paganova (2009) revealed the reliability of leaves and fruit morphological characters in distinguishing *Pyrus pyraeaster* into four varieties. Moreover, Shrestha *et al.* (2012) demonstrated the role of morphological characters in distinguishing five landraces of *Citrus aurantifolia* and subsequently used those characters as the main basis in genotype selection for breeding programs. Morphological characterization on 20 cultivars of Indonesian *Citrus nobilis* by Martasari and Reflinur (2012) highlighted contribution of morphology in recognizing major differences among cultivars.

The use of morphological characters in plant systematics is relevant in any level, even though many molecular techniques has been developed in elucidating relationships among taxa. Morphological characterization is the first step in classification and being the basic information for further analysis using biochemical or DNA markers (Bozokalfa *et al.*, 2009). The important role of morphology is pointed out by Ceolin and Miotto (2011) by asserting that morphology continues to be the main source of information in the recognition of plant taxa, even when DNA barcoding techniques was established for many taxa. In this regard, Serebryanaya and Shipunov (2009) emphasized the need of detail morphological analysis before any taxonomic conclusion could be drawn in responding to population variations, especially when dealing with geographically

separated populations in the context of evolutionary process. Moreover, De Lima *et al.* (2012) states that morphological description provides valuable information for the prediction of genotypic variability. Likewise, morphological characters are very useful in the development of digital database as reference system in the future (Dwari and Mondal, 2011).

Regarding to the implication of morphological variation on the infraspecific classification, the main criteria for recognition of subspecies is the coherence of morphology supported by solid association to geographical distribution (Scheepens *et al.*, 2011; Swanepoel, 2012). When this criterion is not found in the observed samples or populations, then the infraspecific categories of subspecies could not be assigned. By using this criterion, the grouping of pummelo accessions using phenetic approach based on morphological characters was not indicating the existence of taxonomic entities representing subspecies. Instead, the two clusters generated from the phenetic analysis represented two cultivar groups, corresponding to the “Sour and Bitter Pummelo” and “Sweet Pummelo” groups.

In the view of plant systematics, the variability of leaves and fruit characters in pummelo may arise by hybridization or selection processes which resulted in the occurrence of adaptive phenotypic variation. Concerning the selection process during long cultivation history, Fuller and Allaby (2009) suggested that different selective pressures occurred different organs as target of selection. Similar argument was advocated by Mladenovic *et al.* (2012) in their study on morphological characterization of *Lagenaria siceraria* based on multivariate analysis on fruit and seeds morphology. This study revealed that morphological variability on fruit and seeds in *L. siceraria* was resulted from selection process based on preferences on particular shapes and size of fruit. In this regard, Singh *et al.* (2013) noted that shape of organ is a morphological feature considered to be a stable taxonomic character and thus is suitable to be used in practical classification. Referring to previous studies, it could be asserted that pummelo leaves and fruit are two organs representing selection target and hence show high degree of variability. Another important point deduced from this study was that the use of leaves and fruit morphology for the recognition of cultivar groups in pummelo is considerably effective as their features are easily identified and not affected by differences of habitat and growth seasons.

## 5. CONCLUSION

The main taxonomic conclusion arising from this study is that two discrete clusters are recognizable among the 60 pummelo accessions, assigned here as two different cultivar groups. Each group is representing highly variable assembly of cultivars and accessions deserve attention for their conservation. Phenotypic variability of pummelo accessions was adequately fulfilling criteria as gene pools, as they represented accessions from different geographical distribution areas in Indonesia. They warrant consideration for conservation properly in order to protect loss of potential plant resources.

## 6. ACKNOWLEDGEMENT

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