




Morphological characterization of *Coelogyne* spp for germplasm conservation of orchids

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ABSTRACT

Orchid is one of the ornamental plant that has a high aesthetic value. Efforts to increase the quality of orchids can be done by genetic improvement through crossing. The first step to success in orchid crossing requires information on morphological characters of the prospective parents. This study aims to determine the similarity of six species of natural *Coelogyne* spp based on qualitative morphological characters. Morphological characterization of 6 orchids was carried out based on 30 characters including stem, leaf, flower, pseudobulb, and rooting type. Cluster analysis was carried out with the NTSYSpc program version 2.02i with the UPGMA SimQual function method. The results showed that there were morphological diversities among the six *Coelogyne* spp on the character of the stem, pseudobulb, leaves, flowers and type of roots. Based on the dendrogram on 73% similarity, three major groups were obtained. The first group consisted of *Coelogyne pandurata* and *Coelogyne rumphii*, the second group was *C. massangeana*, *C. mayeriana* and *C. asperata*, the third group was *C. celebensis*. Based on the morphological similarity of 87% there are two pairs of parents that have most successful chance to be crossed, *C. pandurata* with *C. rumphii* and *C. mayeriana* with *C. asperata*.

Keywords: character; cluster analysis; genetic improvement; morphological similarity.

INTRODUCTION

Orchidaceae is one of the largest family of plants, consisting of more than 25,000 species. Orchids are recognized as economically important commodities in the international flower industry, either as cut flowers or potted plants (Chase 2005; Hartati *et al.*, 2017; Kuehnle, 2007). In addition to being used as an ornamental plant, several types of orchids have also been used as food or medicine (Luo *et al.*, 2014).

The existence of natural orchids in their habitat is getting closer to extinction. Therefore increasing the genetic diversity of orchids by crossing is an important action to be taken.

Genetic relationship among cultivars is a very important factor for the success of a plant crossing program. Crossing

between closely related species will increase the chances of success of crossing (Purwantoro *et al.* 2006). On the other hand the further the relationship between the parents, the smaller the success of the crossing (Julisaniah *et al.*, 2008; Xu *et al.* 2010; Xue *et al.* 2010).

Genetic relationships of plants can be studied by using markers as a tool to carry out genetic characterization both molecular and morphological (Kartikaningrum *et al.*, (2002). Morphological markers on plants include roots, stems, leaves, flowers, fruit, and so on. In orchids, the morphological characters of leaves and flowers are characters that are used as markers to distinguish between plant groups (Pangestu *et al.*, 2014).

Rahayu & Handayani (2008) stated that morphological characters are characters that are easily seen and not hidden

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characters, so the variations can be assessed quickly when compared with other characters. Moreover characterization of genetic diversity and relationship between *Dendrobium* orchids is very important for the sustainability of conservation and increasing the usefulness of plant genetic resources (Wang *et al.*, 2009).

Several studies on morphological characterization of orchids have been carried out. One of them is a study to analyze the grouping and genetic relationship of 30 species of *Dendrobium* spp. in India by De *et al.* (2015). Nevertheless, the literature presenting the results of the study of the orchid population is still very low (Bhattacharyya, 2015). In addition, there are no research report that are related to the characterization of the *Coelogyne*, so information about the characteristics of *Coelogyne* spp is still very difficult to obtain.

Characterization can also be used as a basis for phylogenetic studies to determine crop diversification patterns (Freudenstein *et al.*, 2015). In plant breeding, the results of characterization can be used as basic information about diversity and classification that can show the level and relationship between cultivars (Nandariyah, 2010). Characterization of genetic diversity and genetic relationships of orchids is very important for conservation of conservation and increasing the use of plant genetic resources (Wang *et al.*, 2009).

The objective of this study is to determine the similarity of six natural *Coelogyne* spp species based on qualitative morphological characters for selecting the prospective parents of crossing.

MATERIALS AND METHODS

Materials

The materials used in this study were 6 *Coelogyne* spp (Table 1 and Figure 1) which is collection from Bogor Botanical Garden.

Method

The research was carried out by direct morphological observation and documenting plant parts from 6 *Coelogyne* species. Characterization was carried out on the stem, leaves, flowers and roots covering 30 characters using the scoring as done by Balithi, 2007.

Statistical analysis

Data analysis was performed using morphological data scoring from description to binary data. Morphological characters are analyzed by marking there (1) or none (0) for each character produced.

The data obtained were analyzed with the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System) version 2:02 Unweight pair group method with arithmetic method (UPGMA) function SIMQUAL (Qualitative Similarity) and utilized to obtain the genetic similarity matrix using Dice coefficient (Rohlf (1998)), the UPGMA (*Unweighted Pair Group Method using Arithmetic Average*) clustering method was used to construct a dendrogram.

RESULT AND DISCUSSION

Morphological characterization of six *Coelogyne* species, *C. pandurata*, *C. massangeana*, *C. mayeriana*, *C. asperata*, *C. celebensis* and *C. rumphii* covering 30 characters (Balithi, 2007) is presented in the Table 2.

The similarity values based on morphological markers among the six *Coelogyne* orchids are presented in the matrix in Table 3. The matrix shows that the similarity values between *Coelogyne* orchids ranged from 0.67-0.87. The similarity in morphological characteristics between large species reflects the similarity of genetic relationship between one species and the other. The similarity value of 0.87 is found between *C. pandurata* and *C. rumphii*. and *C. mayeriana* with *C. asperata*. Because in this study the target of selecting parents who have genetic similarity is *C. pandurata* as the parent possessing genetic closeness is *C. rumphii*. and *C. mayeriana* with *C. asperata*.

From the characters observed there were differences in morphological properties, namely the flower *C. pandurata* in green with black tongue petal (crown) shape ovate, the number of leaves in the two medium bulb *C. rumphii* yellow tongue brown petal shape straight, the number of leaves on the bulb one.

C. mayeriana has symmetry leaves, curvature lip at the base, longitudinal cross section shape pseudobulb and moderate size pseudobulb. While *C. asperata* has no asymmetry leaves, curves lip in the middle, longitudinal

Table 1: Plant materials and the origin

No	Species	Locality and Habitat	Altitude (m)
1	<i>Coelogyne pandurata</i>	East Kalimantan	100
2	<i>Coelogyne masangeana</i>	West Sumatra	1150– 2100
3	<i>Coelogyne mayeriana</i>	Kalimantan	100
4	<i>Coelogyne asperata</i>	WestKalimantan	320 – 1000
5	<i>Coelogyne celebensis</i>	South Sulawesi	826 – 220
6	<i>Coelogyne rumphii</i>	South Sulawesi	100 – 2000

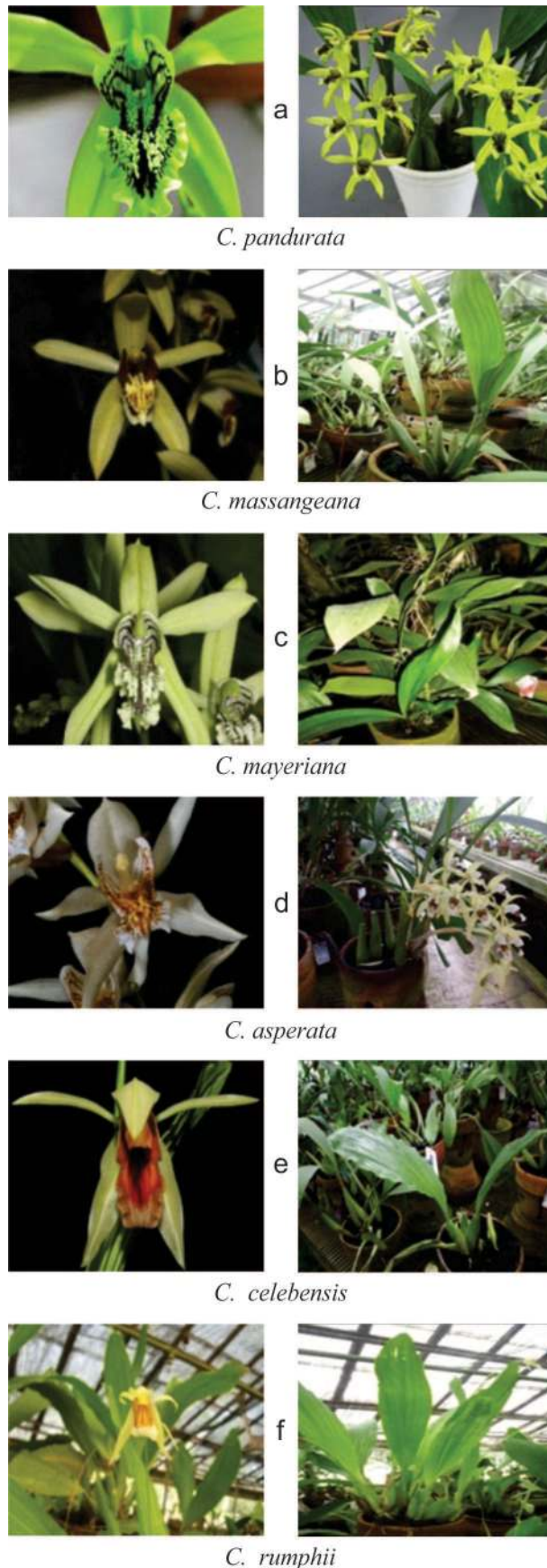


Figure 1: Morphological orchid *Coelogyne* spp: a. *C. pandurata* b. *C. massangeana* c. *C. mayeriana* d. *C. asperata* e. *C. celebensis*, f. *C. rumphii* (Bogor botanical garden Indonesia, 2012).

shape of eye javelin pseudobulb and large size pseudobulb. Pseudobulb has an important role as a storage medium for water, carbohydrates and minerals in orchid (Yang *et al.*, 2016).

The dendrogram of *Coelogyne* spp based on morphology characters is presented in the Figure 2. The dendrogram shows similarities among the six orchids are between 70% - 87%. Therefore the genetic diversity among the six *Coelogyne* spp ranges from 13% -30%. From the six *Coelogyne* species in the similarity of 0.73 produces three groups, the first group consists of *C. rumphii*, and *C. pandurata*, the second group was *C. massangeana*, *C. mayeriana* and *C. asperata* while the third group consists of *C. celebensis*. This is in accordance with the study of Maiti *et al.* (2009) and Khosravi *et al.* (2009) who stated that orchid plants are flowering plants that have a high diversity pattern. While the previous study of Hartati & Darsana (2015) show that there are morphological similarities in several genera of orchids from 50-100%.

Since the closer the parent's genetic relationship the greater the chance of success in crossing (Purwantoro *et al.*, 2006; Julisaniah *et al.*, 2008; Xue *et al.* 2010), this study found that among the 6 species being studied, there are two pairs of parents that have most successful chance to be crossed, *C. pandurata* with *C. rumphii* and *C. mayeriana* with *C. asperata*.

The previous research showed that the dendrogram result indicated a considerable level of the molecular RAPD analysis showed six species forming three clusters with 46% similarity level. First cluster are *C. pandurata*, *C. rumphii* and *C. celebensis*. The second clusters are *C. mayeriana* and *C. asperata* the third cluster is *C. massangeana*.

The genetic range of six species from the *Coelogyne* genus is from 0.23-0.54 (Hartati *et al.*, 2014). Another study identified the same 6 species of *Coelogyne* using molecular ISSR found that they had similarity coefficients ranging from 0.32 to 0.70, meaning that the genetic diversity of the orchid species studied was spread between 30% to 68%. Dendrogram shows that ten ISSR molecular markers are able to classify tree similarity clusters with a similarity coefficient of 0.51. The first cluster consisted of *C. pandurata*, *C. rumphii*, *C. mayeriana*, *C. asperata*, the second cluster was *C. celebensis* and the third was *C. massangeana* (Hartati *et al.*, 2017).

C. celebensis and *C. rumphii*, found in Peninsular Malaysia, Sumatra, Java, Kalimantan, Sulawesi and Maluku. All of these species have pseudobulb unifoliate except *C. celebensis* and *C. asperata*, which can also have two leafy pseudobulbs, another similarity supported by the subgroups of *C. celebensis* and *C. rumphii*, both of which have oval flower shield leaves.

Table 2: Morphological characters of 6 Coelogyne species

Morphology	Species					
	<i>C. pandurata</i>	<i>C. massangeana</i>	<i>C. mayeriana</i>	<i>C. asperata</i>	<i>C. celebensis</i>	<i>C. rumphii</i>
Growth type	sympodial	Sympodial	Sympodial	Sympodial	Sympodial	Sympodial
Leaf shape	oblanceolate	oblanceolate	oblanceolate	oblanceolate	obovate	oblanceolate
Leaf cross section	plicate	plicate	plicate	plicate	plicate	plicate
Shape leaf tip	acute	acute	acuminate	acuminate	acuminate	acute
Leaf edge shape	undulate	entire	entire	entire	sinuate	entire
Leaf surface texture	glabrous	glabrous	glabrous	glabrous	glabrous	glabrous
Leaf symmetry	Simetry	Simetry	Simetry	Not simetry	Simetry	Simetry
Leaf color	Green	Green	Green	Green	Green	Green
Flower shape	Star	Star	Star	Star	Star	Star
Dorsal & lateral sepal shape	lanceolate	oblong	lanceolate	lanceolate	lanceolate	lanceolate
Petal shape	linear	linear	linear	linear	linear	linear
Sepal & petal tip shape	Acute	acuminate	acuminate	acuminate	acuminate	Acute
Callus type on the lips	Simple	Simple	Simple	Simple	Complex	Complex
Lip curvature	At the base	At the tip	At the base	at the middle	at the tip	at the base
Murmur in the labellum	No murmur	No murmur	No murmur	No murmur	No murmur	No murmur
Spur	No spur	No spur	No spur	No spur	No spur	No spur
Flowering position	At the top/shoot	At the base	At the top/shoot	At the top/shoot	At the top/shoot	At the top/shoot
Amount of pollinia	Four	Four	Four	Four	Four	Four
Dorsal sepal color pattern	Prevalent	Prevalent	Prevalent	Prevalent	Prevalent	Prevalent
Lateral sepal color pattern	Prevalent	Prevalent	Prevalent	Prevalent	Prevalent	Prevalent
Petal color pattern	Prevalent	Prevalent	Prevalent	Prevalent	Prevalent	Prevalent
Floral aroma	Smelling	Smelling	Smelling	Smelling	Smelling	Smelling
Pseudobulb longitude section	Circular	Linear	Elliptic	Lanceolate	Elliptic	Elliptic
Pseudobulb cross section shape	Circular	Circular	Elliptic/	Elliptic/	Circular	Circular
Rooting type	Sticky root	Sticky root	Sticky root	Sticky root	Sticky root	Sticky root
Leaf arrangement	Convolute	Convolute	Convolute	Convolute	Convolute	Convolute
Resupinate	No resupinate	Resupinate	Resupinate	Resupinate	Resupinate	No resupinate
Transverse & longitudinal shape of dorsal and petal	Concave	Concave	Convex	Convex	Concave	Concave
Cross section of lip	Flips rather deep	Flips out with a curved end	Flips out with a curved end	Flips out with a curved end	Curved inward with a flipped tip	Flips deeply
Pseudobulb size	Large	Medium	Medium	Large	Medium	Large

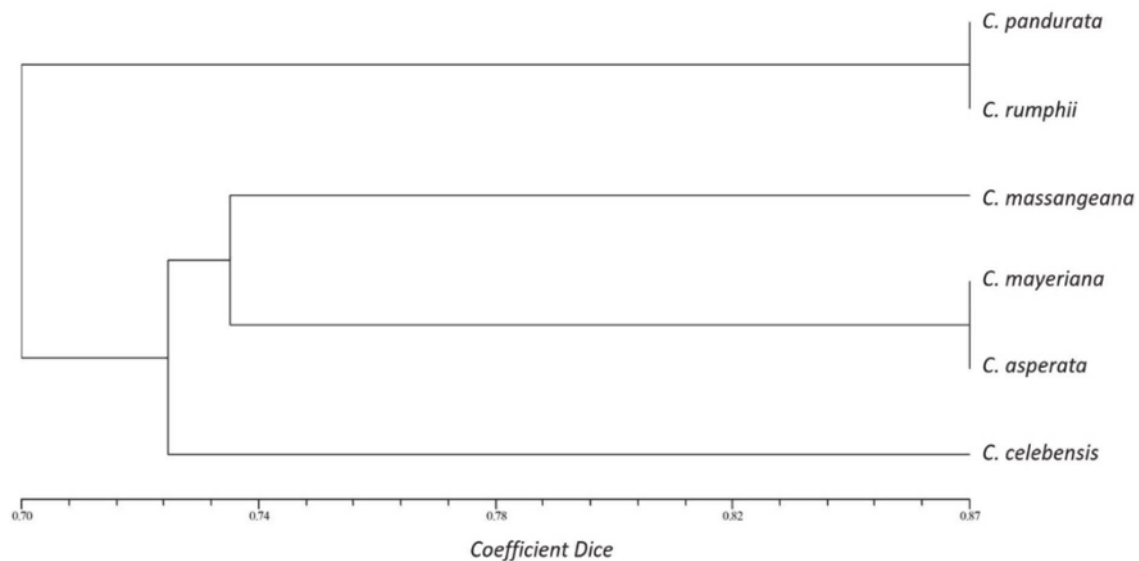
Table 3: Similarity matrix based on morphological markers

	1	2	3	4	5	6
1	1.00					
2	0.70	1.00				
3	0.70	0.77	1.00			
4	0.67	0.70	0.87	1.00		
5	0.67	0.73	0.77	0.67	1.00	
6	0.87	0.70	0.74	0.67	0.73	1.00

Note:

1 = *C. pandurata* ; 2 = *C. Massangeana*; 3 = *C. Mayeriana*; 4 = *C. asperata*

5 = *C. Celebensis*; 6 = *C. rumphii*

**Figure 2:** Dendrogram of *Coelogyne* spp based on morphology characters.

The results of this study show the difference with the results of the description carried out by Gravendeel & Vogel, 2000, namely the number of flowers on each stalk, the stem of the flower, the lower leaf, the blossom of the flower, the petals, the fruit, the lips, the third piece on the lips, the neck monument, stamens and stems that support the female and male genitals.

Clustering results of these six *Coelogyne* orchid species can be used as potential parents in more potential orchid *assemblies*. Lokho & Kumar (2012) stated that characterization data is very useful for resource management and conservation of individual species but also for orchid breeders or farmers.

CONCLUSION

There is a morphological diversity among six natural *Coelogyne* spp in the characterization of stems, pseudobulb, leaves and flowers.

Based on the morphological similarity of 73%, there are three groups were obtained, the first group consists of *C. rumphii*, and *C. pandurata*, the second group was *C.*

massangeana, *C. mayeriana* and *C. asperata* and the third group was *C. celebensis*.

Based on the morphological similarity of 87% there are two pairs of parents that have most successful chance to be crossed, *C. pandurata* with *C. rumphii* and *C. mayeriana* with *C. asperata*.

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