Characterization on the hybrid of Dendrobium bigibbum from Maluku and Dendrobium lineale from Papua, Indonesia

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Abstract. Climate change can potentially lead to reducing biodiversity, including orchids. One of the efforts to increase the biodiversity of orchids is through the crossing. This study aimed to determine the morphological characters of plants resulting from interspecific crosses between Dendrobium bigibbum from Maluku and Dendrobium lineale from Papua, also to compare them with the characters of their parents. In this study, morphological characterization was carried out on 14 accessions, 3 male parent accessions, 3 female parent accessions and 8 F1 offspring accessions. Characterization was carried out based on 30 characters, including stem, leaf, flower, pseudobulb, and root types. The research was conducted in two places, the identification of the parents was carried out at Indonesia Research Institute Botanical Garden Conservation Center in Bogor, Indonesia, while the identification of hybrids was carried out in Karangploso Village, Karanganyar Regency. Cluster analysis was performed using NTSYSpc version 2.02i program with the UPGMA SimQual function method. The results showed differences in leaf and flower morphology between accessions that were observed including size, shape, pattern, and color of the petals and sepals, but the shape and size of the pseudobulbs, color, size and shape. However, leaves tend to resemble one another.

1. Introduction
Orchids are ornamental plants that have high aesthetic value. The flower’s shape and color and other unique characteristics are the main attraction of this plant. Indonesia has many kinds of wild orchids, and Dendrobium genus is the largest orchid genus from Orchidaceae family. This genus is a wealth of Indonesia's genetic resources, abundant in the eastern region, such as Papua and Maluku.

Dendrobium orchids have huge diversity, both in habitat, size, pseudobulb shape, leaves and flower color [1]. This genus’s spread spectrum is broad, from the coast to the mountains, at an altitude of 0–500 meters above sea level with 60–80% humidity. Some species of the genus Dendrobium have a high enough compatibility to be crossed to produce new hybrids. A study by Hartati et al [2] showed the success rate of crossing between female (♀) D. mirbelianum and female (♀) D. lineale, ♀ D. lineale and ♂ D. mirbelianum, ♂ D. lineale and ♂ D. bigibbum, ♀ D. bigibbum and ♂ D. lineale. The percentage of success in all crossing and their reciprocal cross is 100%, except the crossing between ♀ D.lineale and ♂ D. tobaense and its reciprocal cross is not successful. The reciprocal cross is a cross between one individual as a male parent and one as a female parent and the opposite role is that the parent who at the
first cross becomes a male parent acts as a female parent while previously acting as a female parent becomes a male parent.

The occurrence of global climate change is one of the conditions that can threaten the existence of natural orchids. Therefore, it needs efforts to preserve its existence and develop its diversity through plant breeding activities. The original Indonesian Dendrobium orchids that have quite a contrasting appearance are Dendrobium bigibbum from Maluku and Dendrobium lineale from Papua. Several studies have been conducted as an in vitro propagation effort for the two species, including previous research by Tuhuteru et al [3], Paramartha et al [4], Sulasiah et al [5], Sucandra et al [6] and Inkiriwang et al [7]. However, so far, no research has been carried out that provides information on the morphological marker of the two natural orchid species from the eastern region of Indonesia and their crossing results.

According to Pangestu et al [8], morphological marker on plants is a process to determine the phenotypic characters of a plant by observing the stems, fruits, roots, leaves and flowers, that covers the entire morphology of plants and know the genetic relationship between species. The genetics relationship analysis's weakness based on morphology characters is a marker, which is strongly influenced by the environment [9]. Therefore, it is necessary to appropriately characterize agronomic and morphology to facilitate germplasm utilization by breeders [10].

2. Materials and methods
The research was carried out from October 2018 to September 2019. Morphology identification of the orchid parents was conducted in the Plant Conservation Center Botanical Gardens, Indonesia Research Institute (LIPI) Bogor, West Java. It is located in the lowlands of 235-260 meters above sea level (masl). The orchids were observed in the greenhouse with temperatures ranging from 24.9-27 °C and relative humidity of 81-97%. The identification of the hybrids (F1) resulted from crossing was conducted in the collection garden in the village of Plosorejo, Matesih District, Karanganyar Regency at an altitude of ± 461 masl.

The research materials consist of mature plants of Dendrobium bigibbum and Dendrobium lineale, respectively three plants, a Bogor Botanical Garden collection, and the crossing hybrids of Dendrobium bigibbum>Dendrobium lineale and Dendrobium lineale>Dendrobium bigibbum, four plants in each crossing species.

Primary data were collected through observation at mature plants that have been flowering while the secondary data were simply in image documentation. The direct identification of the orchids organs morphology was based on Characterization of Orchid Ornamental Plants Guideline [11]. It consist of 30 qualitative characters, namely the growth type, leaf shape, leaf cross-section, leaf tip shape, leaf edge shape, leaf surface texture, leaf symmetry, leaf color, flower shape, dorsal sepal shape, petal shape, sepal tip shape and petal tip shape, callus type on labium, location of labium indentation, tentacle on labellum, spurs, flower position, number of pollinia, dorsal sepal pattern, lateral sepal pattern, petal pattern, floral scent, pseudobulb size, pseudobulb longitudinal shape, pseudobulb cross-sectional shape, root type, leaf arrangement, resupinate, transverse and longitudinal shapes of sepals and petals, and labium cross-sections.

The data were analyzed descriptively and processed into binary data then it was computed in Numerical Taxonomy and Multivariate Analysis System (NTSYS) ver. 2.02 program. The qualitative coefficient of character similarity between individuals was obtained through Simqual analysis [12]. The level of similarity coefficient value ranges from 0 to 1. The greater the similarity coefficient value between individuals means the closer the genetics relationship.

3. Results and discussion
The qualitative morphological characters of D. bigibbum, D. lineale, F1 of the crossing, D. bigibbum>D. lineale and D. lineale>D. bigibbum are shown in Table 1. Meanwhile, the qualitative character similarity analysis results on 14 individual orchids included both parents and the crossing results and their reciprocal (F1) are shown in the similarity coefficient values (Table 2).
Table 1. Qualitative morphological characters of *Dendrobium* sp.

| No | Morphology characters                        | D. bigibbum | D. lineale | $F_1$: D. bigibbum $>$ $<$ | $F_1$: D. lineale $>$ $<$ |
|----|---------------------------------------------|-------------|------------|-----------------------------|-----------------------------|
| 1  | Growth type                                 | sympodial   | sympodial  | sympodial                   | sympodial                   |
| 2  | Leaf shape                                  | lanceolate  | lanceolate | lanceolate                  | lanceolate                  |
| 3  | Leaf cross section                          | conduplicate| conduplicate| conduplicate                | conduplicate                |
| 4  | Leaf tip shape                              | retuse      | retuse     | retuse                      | retuse                      |
| 5  | Leaf edge shape                             | entire      | entire     | entire                      | entire                      |
| 6  | Leaf surface texture                        | glabrous    | glabrous   | glabrous                    | glabrous                    |
| 7  | Symmetry leaf                               | no symmetry | no symmetry| no symmetry                 | no symmetry                 |
| 8  | Leaf color                                  | light green | light green| dark green                  | dark green                  |
| 9  | Flower's shape                              | round       | horned     | star                        | round                       |
| 10 | Resupinate                                  | yes         | yes        | yes                         | yes                         |
| 11 | Sepal dorsal lateral shape Transverse and longitudinal shape of petal and dorsal | lanceolate | straight | lanceolate                  | lanceolate                  |
|    |                                            | concave     | concave    | concave                     | concave                     |
| 13 | Petal shape                                 | obovate     | straight   | spathulate                  | straight                    |
| 14 | Sepal tip shape                             | acute       | acute      | acute                       | acute                       |
| 15 | Petal tip shape                             | retuse      | retuse     | retuse                      | retuse                      |
| 16 | Type of callus                              | simple      | lamellate  | lamellate                   | lamellate                   |
|    | Location of labellum                         | middle      | middle     | middle                      | middle                      |
| 17 | Tentacle-like on the labellum               | no          | no         | no                          | no                          |
| 18 | Cross section of the labellum               | flipped in  | flipped out| flipped out                 | flipped in                 |
| 19 | Spur                                        | yes         | yes        | yes                         | yes                         |
| 20 | Flowering position                          | tip         | tip        | tip                         | tip                         |
| 21 | Pollinia                                    | 2           | 2          | 2                           | 2                           |
| 22 | Sepal dorsal collor pattern                 | equally     | equally    | equally                     | patterned                   |
| 23 | Sepal lateral color pattern                 | equally     | equally    | equally                     | striped                     |
| 24 | Petal color pattern                         | equally     | equally    | equally                     | equally                     |
| 25 | Floral scent                                | yes         | yes        | yes                         | yes                         |
| 26 | Pseudobulb longitude shape                  | lanceolate  | lanceolate | lanceolate                  | lanceolate                  |
| 27 | Pseudobulb cross section shape              | round       | elliptic   | round                       | elliptic                    |
| 28 | Root type                                   | sticky      | sticky     | sticky                      | sticky                      |
| 29 | Pseudobulb size                             | medium      | medium     | medium                      | medium                      |

Qualitative morphological characteristics identification found 8 out of 30 characters observed (26.6%) shows variations, namely leaf color, flower’s shape, petal’s shape, type of callus, sepal dorsal and lateral pattern, cross-section of the labellum, and pseudobulb cross-section shape (Table 1). This indicates that crossing creates variations in plant performance successfully. The shape and color pattern
of flowers on *Dendrobium* sp. is the most important component because it determines selling value. Crossing between *D. lineale* and *D. bigibbum* produced new shapes and color patterns on flowers' sepals and petals. This gives hope to create new varieties by utilizing the diversity of flowers.

3.1. The genetics relationship between *D. bigibbum* and F1 of the crossing *D. lineale*<<*D. bigibbum*  
Table 2. shows the similarity coefficient between *D. bigibbum* and F1 of *D. lineale*<<*D. bigibbum*. The similarity coefficient of *D. bigibbum* was equal to 0.83 and F1 of *D. bigibbum*<<*D. lineale* was equal to 0.85. The genetic relationship of various plant species is beneficial as source of information in determining the selected parent of two closely related species. This can be used as a reference in carrying out hybridization to get new varieties.

There were several differences in morphological characters between *D. bigibbum* and F1of *D. lineale*<<*D. bigibbum* and between *D. lineale* and F1 of *D. bigibbum*<<*D. lineale*, namely in the shape of flowers, plant size, shape and pattern of petal colors, shape and sepals (Table 1). The similarity between accessions can be seen in the shape and pseudobulbs, which were categorized in medium size. The larger pseudobulb size means the greater orchid’s resistance to drought. The presence of pseudobulbs can help reduce moisture loss in the leaves during drought and stress by distributing water reserves from pseudobulbs to leaves [13]. The fourth of accession of orchids with a green leaves color and the leaves' size and shape are almost the same.

*Dendrobium bigibbum* and F1 of *D. lineale*<<*D. bigibbum* morphological characteristics were found in common flowers. The morphological characteristics were round, with *lanceolate* sepals, with pointed petals tip (*acute*) and blunt notched petals tip (retuse) slightly, but both have differences in petals, which is oval (*obovate*) and ribbon/straight (*linear*). Andri *et al* [14] stated that the diversity and distinctive characteristics of the color and shape of orchids are special attractions that need to be developed in order to increase the selling value of orchids. Orchid flowers have their peculiarities to attract birds or insects for pollination. Orchids pollination is mainly assisted by birds or insects attracted by the colors, shapes, and various fragrances produced by the flowers [15].

### Table 2. Similarity coefficient matrix for *Dendrobium* sp. based on the qualitative morphological characters

| No. | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 |
|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1   | 1.00 |   |    |    |    |    |    |    |    |    |    |    |    |    |
| 2   | 1.00   | 1.00 |    |    |    |    |    |    |    |    |    |    |    |    |
| 3   | 1.00   | 1.00 | 1.00 |    |    |    |    |    |    |    |    |    |    |    |
| 4   | 0.84   | 0.84 | 0.84 | 1.00 |    |    |    |    |    |    |    |    |    |    |
| 5   | 0.84   | 0.84 | 0.84 | 1.00 | 1.00 |    |    |    |    |    |    |    |    |    |
| 6   | 0.84   | 0.84 | 0.84 | 1.00 | 1.00 | 1.00 |    |    |    |    |    |    |    |    |
| 7   | 0.82   | 0.82 | 0.82 | 0.85 | 0.85 | 0.85 | 1.00 |    |    |    |    |    |    |    |
| 8   | 0.82   | 0.82 | 0.82 | 0.85 | 0.85 | 0.85 | 1.00 | 1.00 |    |    |    |    |    |    |
| 9   | 0.82   | 0.82 | 0.82 | 0.85 | 0.85 | 0.85 | 1.00 | 1.00 | 1.00 |    |    |    |    |    |
| 10  | 0.82   | 0.82 | 0.82 | 0.85 | 0.85 | 0.85 | 1.00 | 1.00 | 1.00 | 1.00 |    |    |    |    |
| 11  | 0.82   | 0.82 | 0.82 | 0.79 | 0.79 | 0.79 | 0.77 | 0.77 | 0.77 | 0.77 | 1.00 |    |    |    |
| 12  | 0.82   | 0.82 | 0.82 | 0.79 | 0.79 | 0.79 | 0.77 | 0.77 | 0.77 | 0.77 | 1.00 | 1.00 |    |    |
| 13  | 0.85   | 0.85 | 0.85 | 0.82 | 0.82 | 0.82 | 0.80 | 0.80 | 0.80 | 0.80 | 0.97 | 0.97 | 1.00 |    |
| 14  | 0.82   | 0.82 | 0.82 | 0.79 | 0.79 | 0.79 | 0.77 | 0.77 | 0.77 | 0.77 | 1.00 | 1.00 | 0.97 | 1.00 |

*Description:*  
1. *D. bigibbum* (1), 2. *D. bigibbum* (2), 3. *D. bigibbum* (3), 4. *D. lineale* (1), 5. *D. lineale* (2), 6. *D. lineale* (3), 7. *D. bigibbum*<<*D. lineale* (1), 8. *D. bigibbum*<<*D. lineale* (2), 9. *D. bigibbum*>>*D. lineale* (3), 10. *D. bigibbum*>>*D. lineale* (4), 11. *D. lineale*<<*D. bigibbum* (1), 12. *D. lineale*<<*D. bigibbum* (2), 13. *D. lineale*<<*D. bigibbum* (3), 14. *D. lineale*<<*D. bigibbum* (4).
3.2. The genetics relationship between D. lineale and F1 of D. bigibbum

The similarity coefficient value between D. lineale and F1 of D. bigibbum < D. lineale was 0.85. Both accessions have differences in the cross-section of pseudobulb, which are oval (elliptic) and round (circular), sepal of D. lineale is ribbon/straight (linear), sepal and petals colors are patterned with a purplish-white base color, while F1 of D. bigibbum < D. lineale has lanceolate sepal shape and petal shape resembles a spoon (spathulate). The morphological diversity observed at four accessions as a result of genetics influence on the parents. Besides, the diversity of Orchidaceae family’s flowers can be caused by natural selection and adaptation process to natural habitat and factors beyond induced genetic changes that facilitate their evolution [16]. The color that appears on plant parts, especially flowers, is caused by plants' pigments [17]. In general, flower coloring between reddish-orange to red is influenced by anthocyanins and carotenoids as in Chrysanthemum genus, between red and purple influenced by anthocyanins and green to yellow is influenced by chlorophyll [18]. Petals with intense/bright colors have high anthocyanin levels, while pale ones have high amounts of chlorophyll content [19].

Figure 1 presents a dendrogram based on qualitative morphological characteristics that shows all four Dendrobium sp. and had a resemblance up to 0.8 or 80%. Coefficient of dendrogram (1.0) is divided into two parts. The first part consists of D. bigibbum and F1 of D. lineale < D. bigibbum, while D. lineale and F1 of D. bigibbum < D. lineale are separated from other Dendrobium sp. Similarity between accessions shows genetic relationship between the tested accessions [20]. Four observed Dendrobium have a close genetic relationship, so they have a great potential for crossing (Figure 1). F1 of D. lineale < D. bigibbum has a close genetic relationship with D. bigibbum, while F1 of D. bigibbum < D. lineale has a close genetic relationship with D. lineale.

One of efforts to improve orchids quality can be done through genetic improvement by crossing [21]. Selection of parents quality is very important factor in determining the success of hybridization program. The greater the genetic diversity will increase selection effectiveness, so new varieties can be obtained

| Description: | DB = D. bigibbum | DBxDL = D. bigibbum < D. lineale | DL = D. lineale | DLxDB = D. lineale < D. bigibbum |
|--------------|------------------|----------------------------------|----------------|----------------------------------|

![Dendrogram](image-url)
with desired superior characteristics, especially can adapt to new environmental conditions outside of their natural habitat.

4. Conclusion
There were differences in flowers morphology among Dendrobium bigibbum, Dendrobium lineale (hybrids of crossing between Dendrobium bigibbum > Dendrobium lineale and Dendrobium lineale >< Dendrobium bigibbum), which included size, shape, pattern and color of petal and sepal, but the shape and size of pseudobulb, color, size and shape of leaves tend to resemble each other. This research succeeded in creating new individuals of cross-bred orchids that are expected to increase orchids' biodiversity.

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