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Morphological Characteristics and Pollinia Observation of 10 Indonesia Native Dendrobium Orchids

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Abstract :

Characterization of native orchid (species) in Indonesia is important for preservation of germplasm and selection process to obtain dominant character of orchids as potential parental. Morphological characterization is a method to obtain scientific information in phenotypic manner. Characterization of plants was carried out on 10 native orchids of Dendrobium genus to obtain specific character. Previous observation has been done in Phalaenopsis genus to identify their morphological characters. Characterization process based on UPOV guidebook and morphological observation of pollinia was also carried out to obtain information about the structure and physiology of pollinia. Observing pollinia in this research is for gathering scientific information about the shape, size, color and number of pollinia for orchids reproduction system. The results of morphological characterization on 10 native orchid belonging to spatulata section in Indonesia showed several qualitative characters such as flowering position, cross section of leaf, pseudobulb firmness, and leaf arrangement in D. Canaliculatum was different from 9 other native orchid. Morphological pollen of 10 native orchid shows the diversity structure of pollinia and the physiological conditions of mature pollen which are ready to pollinate are pollinia with a golden yellow colored. Knowledge and information about parental relationships is the main point for developing ornamental plant breeding programs. Selection of native orchids as potential elder is important where selected elders will be used as a source of genetic diversity. The results of this study are expected to provide scientific information and phenotypic selection for the development of hybrid orchids.

Keywords: Morphological charaterization, Pollinia observation, Dendrobium.

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1. Introduction

Orchidaceae is the second largest flowering plant family, consist of 20,000 species and 850 genera. The level of Orchid's diversity increasing towards tropical countries, where the epiphytic orchids dominate nearly 73% of the total species. The Orchidaceae family provides several type of differentiation between population at the genetic level [1]. Orchidaceae (orchids) are ornamental plants that have high level of diversity. *Dendrobium* is one of the genus that's widely distributed in Indonesia. *Dendrobium* were divided into 20 section. One of the famous section in Indonesia is *Spatulata (Certaobium)* section [2]. *Spatulata* section has unique characteristic of flower perianth, the

twisted petal like a horn of antelope. The main character of Antelope orchids (*spatulata* section) is sepals and petals formation tend to twist. In addition, having a pseudobulb shape that tends to erect [2]. Natural orchids or orchid species are assets of the Indonesia that need to be preserved for the succession of ornamental plant breeding [3] and the preservation of germplasm [4].

Diversity in orchids can be studied from morphological and anatomical aspects. Morphological observations are easier to do because of the dominant character can be found in several part of the plant [5]. Morphological characterization activities are needed for the variety selection of native orchid germplasm. Morphological characterization includes the process of identification and documentation aimed to obtain plant phenotypic characters [4]. Observation of morphological characters can be done on all parts of the plant which include stems, flowers, leaves, roots, seeds, and fruit [6]. Leaf and flower characterization was carried out by [7] on several species of *Phalaenopsis* orchid accessions to obtain morphological information and data.

Studies related to orchid pollen are also interesting things to do. The morphological structure of orchid pollen is scientific information for the field of taxonomy and ecology [8]. *Pollinia* is an evolutionary form of the aggregation process of pollen which is known to only originate from two families, Orchidaceae and Apocynaceae. Orchids show various types of pollen aggregation. Studying orchid pollen can enrich knowledge related to the evolution of reproductive traits, increase the allocation of female reproductive organs, and pollination with deceptive patterns. Pollen aggregation affects all phases of reproduction from pollen to seed production [9].

2. Materials and Methods

The research materials were 10 native *Dendrobium* in Indonesia, belonging to spatulata section (figure 1). The study was conducted at DD Orchids Nursery, Batu, East Java. Morphological characterization using the UPOV guidelines developed by the Indonesian Ornamental Plants Research Institute. UPOV guidelines contain several descriptors and scoring for identifying and determining morphological parts starting from leaves, pseudobulb, flower perianths and roots of the orchids. Pollen sampling were taken by using characterization equipment and placed in the icebox. Samples were then observed using a digital microscope at the Tissue Culture Laboratory, Faculty of Agriculture, University of Jember.

3. Results and Discussion

Characterization is part of germplasm conservation activities aimed at inventorying plant morphology. Plant morphology has a function as differentiator between accession, comparison of genetic diversity and identification of varieties [10].





Figure 1. Native orchid of *Dendrobium* genus, spatulata section. (a) *D. antennatum*, (b) *D. canaliculatum*, (c) *D. discolor*, (d) *D. racieanum*, (e) *D. strebloceras*, (f) *D. lasianthera*, (g) *D. leporinum*, (h) *D. lineale*, (i) *D. sylvanum*, (j) *D. strepticeros*.

Characterization result of 10 native Indonesian orchids belonging spatulata section, showed that *D. canaliculatum* have several morphological characters such as flowering position, pseudobulb uprightness and leaf cross section that differ from 9 other orchids. The flowering position on *D. canaliculatum* was at the tip-position, while the other 9 species are on side-position. The pseudobulb *D. canaliculatum* was semi-hanging with 9 orchids and the other species were semi-erect. The results of leaf characterization, fruit type and root type in 10 *Dendrobium* orchid species listed in table 1 and table 2.

Leaf characters in 10 species of orchids show that there were morphological diversity in leaf shape, leaf tip shape, leaf surface texture (figure 2) and leaf arrangement (figure 3). Morphological diversity was also found in the root types of the 10 orchid species studied. The results of the characterization of flowers, sepals, petals and *labellum* (lips) were in table 3, table 4, table 5 and table 6.

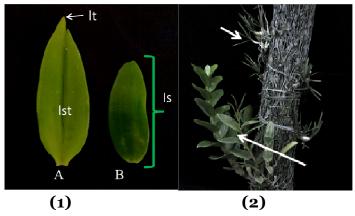


Figure 2. Example of leaf shape (ls), leaf tip shape (lt), leaf surface texture (lst) in A: *Dendrobium antennatum*, B: *Dendrobium strepticeros*. Figure 3. Example of leaf arrangement in A: *Dendrobium canaliculatum*, B: *Dendrobium lasianthera*.

		Morphological Characters				
	Species	Leaf shape	Leaf tip shape	Leaf symmetrical	Leaf texture	Leaf arrangement
1	Dendrobium antennatum	ovate	acuminate	asymmetric	glabrous	duplicate
2	Dendrobium canaliculatum	lanceolate	acute	symmetric	glabrous	convolute
3	Dendrobium discolor	ovate	acute	symmetric	glabrous	duplicate
4	Dendrobium lasianthera	obovate	obtuse	symmetric	farinose	duplicate
5	Dendrobium leporinum	ovate	acute	symmetric	glabrous	duplicate
6	Dendrobium lineale	eliptic	acute	symmetric	glabrous	duplicate
7	Dendrobium racieanum	ovate	acute	symmetric	glabrous	duplicate
8	Dendrobium strebloceras	eliptic	acute	symmetric	glabrous	duplicate
9	Dendrobium strepticeros	ovate	acute	symmetric	glabrous	duplicate
10	Dendrobium sylvanum	eliptic	acute	asymmetric	glabrous	duplicate

Table 1. Result of leave characterization of 10 native Dendrobium orchids, spatulata section.

Table 2. Results of root and fruit characterization in 10 native Dendrobium orchids, spatulata section.

No	Second	Morphological Characters		
INO	Species	Root type	Fruit type	
1	Dendrobium antennatum	Aerial roots	capsula	
2	Dendrobium canaliculatum	Aerial roots	capsula	
3	Dendrobium discolor	Sticky roots	capsula	
4	Dendrobium lasianthera	Sticky roots	Capsula	
5	Dendrobium leporinum	Aerial roots	Capsula	
6	Dendrobium lineale	Sticky roots	Capsula	
7	Dendrobium racieanum	Sticky roots	Capsula	
8	Dendrobium strebloceras	Sticky roots	Capsula	
9	Dendrobium strepticeros	Aerial roots	Capsula	
10	Dendrobium sylvanum	Aerial roots	Capsula	

Table 3. Results of flower characterization of 10 native Dendrobium orchids, spatulata section.

No	Stragios	Morphological characters				
INO	Species	Flowering type	Stalk Stiffness	Sepal and petal position		
1	D.antennatum	racemose	upright	Straight		
2	D.canaliculatum	racemose	upright	Recurving		
3	D. discolor	racemose	Hanging rod	Incurving		
4	D.lasianthera	racemose	upright	Incurving		
5	D.leporinum	racemose	upright	Recurving		
6	D.lineale	racemose	Horizontal	Straight		
7	D.racieanum	racemose	upright	Recurving		
8	D.strebloceras	racemose	upright	Incurving		
9	D.strepticeros	racemose	Hanging rod	Recurving		
10	D.sylvanum	racemose	Horizontal	Stretched		

NT	Species		Morphological Character				
No		Flower buds	Petal arrangement	Flower position	Ovary length	Flower type	
1	D.antennatum	medium	Opened petal	All directon	medium	Horn type	
2	D.canaliculatum	Minimum	Opened petal	Two direction	short	Star type	
3	D.discolor	maximum	Opened petal	All directon	medium	Curl type	
4	D. lasianthera	medium	Opened petal	All directon	medium	Horn type	
5	D. leporinum	medium	Opened petal	Two direction	medium	Horn type	
6	D. lineale	Maximum	Opened petal	Two direction	short	Star type	
7	D.racieanum	medium	Opened petal	All directon	short	Horn type	
8	D.strebloceras	maximum	Opened petal	All directon	medium	Horn type	
9	D.strepticeros	minimum	Opened petal	All directon	medium	Horn type	
10	D.sylvanum	maximum	Opened petal	All directon	short	Star type	

The results of flower characterization on 10 species of orchids indicate that 10 *Dendrobium* orchids belonging to spatulata section have *racemose* flowering type, have open petal structure and there was a resupination process. The shape of the flower consists of star-shaped, curly-shaped and horned-shaped. Resupination is a natural physiological process that occurs in several orchid genera. Resupination is an apomorphic character in Orchidaceae's taxonomy. Resupination was rotation process of inferior ovary so that the *labellum* changes position and occupies the bottom of the ovary [11].

Table 4. Results of sepal characterization of 10 native Dendrobium orchids, spatulata section.

Na	Neme mesier	Morphological Characters			
No	Nama spesies –	Sepal shape	Sepal tip shape	Sepal cross-section	
1	D.antennatum	ovate	acute	flat	
2	D.canaliculatum	Tranverse elliptic	truncate	flat	
3	D.discolor	linear	acute	flat	
4	D.lasianthera	ovate	acute	flat	
5	D.leporinum	Tranverse elliptic	acute	flat	
6	D. lineale	ovate	truncate	flat	
7	D. racieanum	ovate	acute	flat	
8	D.strebloceras	ovate	obtuse	flat	
9	D.strepticeros	ovate	acute	flat	
10	D.sylvanum	ovate	truncate	flat	

Table 5. Results of petal characterization of 10 native Dendrobium orchids, spatulata section.

No	Spacios	Morphological Characters				
INO	Species -	Petal shape	Petal tip shape	Petal cross-section	Resupination	Color number
1	D. antennatum	Linear	acute	flat	weak	two
2	D. canaliculatum	Jorong	retuse	flat	weak	three
3	D.discolor	Ovate	acuminate	flat	strong	two
4	D.lasianthera	linear	acute	flat	strong	two
5	D. leporinum	Linear	acute	flat	Middle	two
6	D.lineale	Rhombic	acuminate	flat	weak	three
7	D. racieanum	Linear	acuminate	flat	middle	two
8	D.strebloceras	ovate	Tumpul	flat	strong	three
9	D. strepticeros	Linear	acute	flat	strong	two
10	D.sylvanum	Rhombic	acuminate	flat	weak	one

Morphological diversity of 10 species of orchids in this study were found in the characteristics of sepal, petal, sepal tip, petal tip, number of colors, color, petal twisting, and *labellum* characters. The petal twisting character is a characteristic of the Spatulata *Dendrobium* section. Weak petal twisting were found in the *D. antennatum*, *D. lineale*, *D. sylvanum* and *D. canaliculatum*. Medium petal twisting were found in *D. leporinum* and *D. racieanum*, while strong petal twisting were found in *D. lasianthera*, *D. strepticeros*, *D. strebloceras* and *D. discolor*. 10 species of orchid have rounded and *labellum* shape, side shape of *labellum* were wide-trapezium, triangular to ovoid, middle *labellum* were rhombic and trapezium in shape, *labellum*'s curve located in the middle and at the ends, *labellum*'s pattern were flattened, striped, until edged. The following figure is the morphology of the *labellum* of one of the orchid species, *D. lineale* (figure 4).

Table 6. Labellum (lip) characterization results in 10 native Dendrobium orchids, spatulata section.

No	Species -		Morphological Cl	naracters
INO		Labellum shape	side shape	middle piece shape
1	D.antennatum	elliptic	trapezium	Rhombic
2	D.canaliculatum	elliptic	trapezium	elliptic
3	D.discolor	elliptic	trapezium	elliptic
4	D.lasianthera	rounded	trapezium	Rhombic
5	D. leporinum	rounded	triangular	Rhombic
6	D. lineale	elliptic	trapezium	Rhombic
7	D.racieanum	elliptic	trapezium	elliptic
8	D.strebloceras	rounded	trapezium	Rhombic
9	D.strepticeros	rounded	ovate	Rhombic
10	D.sylvanum	elliptic	trapezium	Rhombic

No	Success		Morphological Characters			
INO	Species	Middle chip pattern	Side chip pattern	Curving positing		
1	D. antennatum	striped	flat	middle		
2	D. canaliculatum	striped	striped	middle		
3	D.discolor	flat	flat	tip		
4	D.lasianthera	striped	flat	tip		
5	D. leporinum	patterned	flat	middle		
6	D.lineale	patterned	patterned	middle		
7	D.racieanum	striped	flat	tip		
8	D.strebloceras	striped	bertepi	middle		
9	D.strepticeros	flat	flat	middle		
10	D.sylvanum	patterned	patterned	middle		

Several sections that were used to become the parental are the Ceratobium/Spatulata, Palaenanthe, Callista, Latourea, and Eugenanthe sections. Narrow petals and sepals, twisted petal, some species have long petals such as antelope horns are the most prominent characters in the spatulata section [12]. Based on the results of the characterization, morphological data were obtained that high morphological diversity was found in the character of the *labellum*.

Labellum was special characters of the Orchidaceae family. Labellum has high diversity of shapes and colors. The diversity of colors in orchids is a focus area for plant breeders to produce new hybrids through crossing. The color of orchids was determined by anthocyanin and carotenoid pigments [13]. Each species in the Orchidaceae family has different labellum characters. Labellum was a modified petal with a variety of unique shapes. Paphiopedilum rungsuriyanum orchid has a labellum that resembles a helmet and V-shaped with a brownish color [14]. Cypri*pedium reginae* orchid has a funnel-like *labellum*, predominantly white in color, spots and red lines in magenta [15].

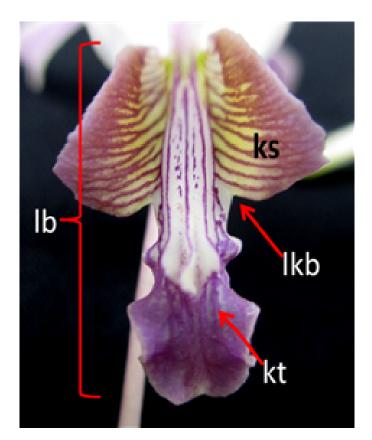


Figure 4. *Labellum* morphology in *D. lineale* orchids. lb = labellum (lip), ks = side-chip, lbb = curve of the labellum, kt = middle-chip.

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Hidayati *et al.* [2] reported that 20 orchid species of the *Dendrobium* genus showed the results of cluster analysis using *labellum* characters were more accurate, so it was concluded that the main differences in orchid species tested were based on their *labellum* character. *Labellum* formation is controlled by special genes that could encode the shape and color of the *labellum*. Apriyanti *et al.* [16] states that the results of orchid characterization based on *labellum* characters can improve the accuracy in identification using cluster analysis.

According to Buragohain et al. [17], the morphology of orchids is very complicated. Female reproductive organs in orchids were called columns, at the apical there was an anther cap. Inside the anther cap contained of pollen called *pollinia* and *pollinaria* (figure 5). Stigma is in the sub-apical column called rostellum. Pollination of orchids occurs when pollinia and pollinaria enter rostellum. The structure of the column and pollinia forms a typical breeding system for orchids called gynostemium [18]. Pollinia was a cohesive mass of many pollen grains released as a unit of anthers. Pollinia is the most evolved form of pollen aggregation. In Orchidaceae, the pollen grains aggregated into one unit because of the absences of wall bridge between nexine and intine layer of pollen wall. Those mechanism allowing for pollen to aggregate, forming "pollinia" as a pollen in family Orchidaceae [9].

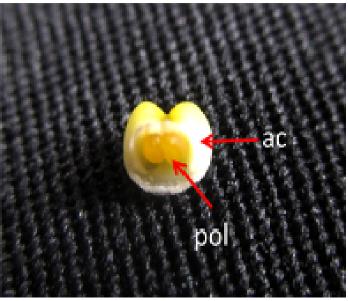


Figure 5. Structure of male orchid reproductive organs consisting of anther cap and *pollinia*. ac = anther cap, pol = *pollinia*.

Pollinia from 10 species of orchids were taken when all flowers are in perfect bloom (*anthesis*). Based on the results of morphological observations through a microscope, 10 pollinia orchid species have different shapes and sizes. *Pollinia* which was ripe and ready to be polished in golden yellow. *Pollinia* from most orchids has a whole and round shape. These polylines are called "wax" or "hard" *pollinia*. The observations are in Figure 6.

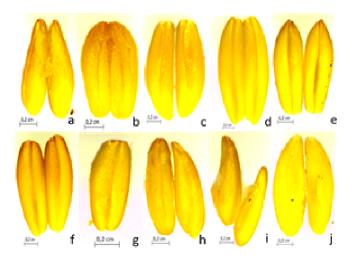


Figure 6. *Pollinia* morphology 10 Orchid species of the *Dendrobium* genus section spatulata. (a) *D. antennatum*, (b) *D. canaliculatum*, (c) *D. discolor*, (d) *D. lasianthera*, (e) *D. leporinum*, (f) *D. lineale*, (g) *D. racieanum*, (h) *D. strebloceras*, (i) *D. strepticeros*, (j) *D. sylvanum*.

The advantage in studying *pollinia* was the physiological function for the orchid itself. *Pollinia* is a pollen aggregation that affects pollen grain dispersion on stigma, *pollinia* is a single pollen contributor for each stigma, which can influence the level and nature competition in the formation of pollen tubes into the ovules when fertilization occurs. *Pollinia* can improve the ability of the ovaries to multiovulate so that the chances of the emergence of seeds in the orchids more and more. Some *pollinia* have additional structures that characterize taxonomy and diversification [9].

Pollinia were important sample in species identification. The process of identifying orchids can use DNA extracted from *pollinia*. The development of the Polymerase Chain Reaction (PCR) and DNA sequencing became revolutionary breakthroughs in the field of biology, specifically plant systematics. The combination of cladistic methodology and molecular tools enables the elaboration of large data matrices that can be carried out with the help of appropriate computer programs, so the results are supported statistically and become more accurate [8]. Studies related to the collection and storage of pollinia samples, as well as molecular level analysis techniques are very important for research contributions in Orchids. Molecular markers can provide a more accurate picture of relationship between species and distant relatives, because the analysis of DNA as a genetic material is not influenced by environmental conditions.

The Characterization result of 10 Native *Dendrobium* orchids belonging to *spatulata* section in Indonesia can be used as scientific information for many requirements in cross-breeding activity, propagation, conservation of germplasm and genetic improvement.

4. Conclusion

Ten (10) native orchids of *Dendrobium* belonging to spatulata section have morphological diversity in several parts such as leaves, roots and flowers especially *labellum*. The overall results of morphological characterization are scientific information that useful for researchers to produce hybrid orchids through selection of orchid species as elders in crossing. *Labellum* is a unique part of orchid's flower that can be used as a character of selection and differentiation between species. Pollinia morphological observations showed that pollinia was in a mature, golden-yellow color and had a variety of structures and sizes in the 10 native orchids observed.

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