Abstract

Alocasia alba Schott is a member of Macrorrhizos group from Aroid family that has conserved in Bali Botanic Garden. On its development, the collections showed varied morphological diversity on leaves and flowers. The aim of this study is to fill the knowledge gap in morphology and anatomy of the species A. alba and to know the phenotypic variation in this species. A total of eight A. alba accessions from Java, Bali and West Nusa Tenggara were observed in morphological and anatomical characters. The result showed that the eight accessions of A. alba have some variations in morphological and anatomical characters. These variations might be caused by genetic factors that resulted from plant adaptation to the different environments.

Keywords: Aroid, environment, leaves, phenotypic, stomata

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Introduction

Alocasia is one of plant group which is very popular among ornamental plant hobbyists and plant breeders. The genus has variety in leave shapes and colors, potentially for exotic plants breeding. Alocasia have estimated 121 species that spread around the world, but only 78 species have been described (Boyce & Croat 2011). The Alocasia distributions and diversities in Indonesia remain unknown. However based on herbarium tracking and field observation, it is estimated about 36 Alocasia species origin from Indonesia (unpublished data). This number may change if the study about Alocasia diversity in its nature habitat is increased. Hay (1998) grouped Alocasia according to similarity on its special character Alocasia i.e Puber, Scabriuscula, Princeps, Macrorrhizos, Longiloba and Cuprea group.

Alocasia alba Schott is a member of Macrorrhizos group with large figures and leaves (Figure 1) which first described by Schott based on cultivated plant from Malesiana region. Botanist identified that A. alba is originated from Java (Hay 1998). Exploration biodiversity in some regions found the new distribution of A. alba in Bali, Lombok (Kurniawan et al. 2013) and Lampung (Mustaqim & Setiawan 2019). In Bali, A. alba has a wide distribution from altitude 196 - 1300 m asl on humus to sandy land.
Figure 1. Alocasia alba Schott (A) Plant (B) Flower (Photograph by I Gede Wawan Setiadi)

Bali Botanic Garden as ex situ conservation institution of Indonesian Institute of Sciences, has conserved A. alba collected from Java, Bali and West Nusa Tenggara. On their development, the collections showed varied morphological diversity on leaves and flowers. Kurniawan et al. (2013) reported about the variation on flower structures and leave shapes in A. alba. Similar research on collected Begonia areolate in Cibodas Botanic Garden from various regions, showed the diversity on their leaves while maintained in the same environmental conditions (Efendi et al. 2020). This showed the genetic influence in plant collection is still remaining although being cultivated away from its natural habitat.

There were less study on phenotypic variation in morphology and anatomy in Alocasia genus. Only two reported articles on Alocasia macrorrhizos (L.) G. Don. such as morphology and reproductive characteristic in Vanuatu (Garcia et al. 2008) also on morphology, anatomy and isozyme variation in Central Java (Suratman et al. 2016). Meanwhile, the morphological and anatomical character research has very important aspect as the parameter to determine diversity level in A. alba. Morphological character often used to represent and identified intra-species together with phenotypic variation because they are fast, simple and inexpensive (Jingura & Kamusoko 2015; Suratman et al. 2016). Anatomical character also useful for systematic study, species identification and solve the taxonomic problem (Chikmawati 2013). The aim of this study is to fill the knowledge gap in the morphology and anatomy of species A. alba and to understand the phenotypic variation in this species.

MATERIALS AND METHODS

Materials

A total of eight Alocasia alba Schott accessions from, Java, Bali and West Nusa Tenggara were observed (Table 1, Figure 2). They were planted in Bali Botanic Garden (BBG) after one year acclimatization in nursery and the plant growth was good. BBG is situated in mountain area at 1,250-1,400 m asl so that the temperature was relatively low.
Methods
Observation of morphology characters was carried out by direct observation of both vegetative and generative characters and character state. The observation of characters included the shape and color of petiole, leaf, peduncle and flower. The observation of character states included the plant height, petiole, peduncle, leaf length and the length of each zone of flower. The plant leaves that used as the main research were the second or third leaves from the top. The color of each part of plants was identified by RHS (Royal Horticultura Society) Color Chart.

Anatomy of leaves anatomy was obtained with modified paraffin-tert-butanol method (Sass 1951). The sections were stained with safranin and fast green. There are two methods for epidermal character observation. We used HNO$_3$ solution (Cutler et al. 2007) to obtain leaf surface and to measure length, width of stomata and simple nail varnish to examine stomatal density (number of stomata/mm$^2$ leaf area) on both abaxial and adaxial surfaces.
RESULTS AND DISCUSSION

Morphological analysis

Morphological vegetative characters, both quantitative and qualitative characters, showed several variations (Table 2). All quantitative characters showed the differences in plants height, petiole and sheath length, leaf length and width, posterior costae diverging, primary lateral vein, peduncle and spathe length, spadix and stipitate length, female zone, male zone, also sterile interstice zone and appendix length.

These color variations also occurred in some of plant parts i.e. in petioles, pattern or line in petioles, leaf colors, axillary glands, peduncles, spathes and each zone in spadix. Some plant characters might have or not patterns or line of petiole. This absence of pattern only found in *A. alba* accession from West Nusa Tenggara. Mostly, leaf characters of accessions from those three locations were similar, but all accessions showed different leaf forms. (Figure 3). Some *A. alba* leaves from Bali accession have suborbicular-sagittate shape, unite posterior lobe, and lanceolate inner-side posterior. *A. alba* from Java accession, has leaf edge which has sinuate character, otherwise West Nusa Tenggara has slightly undulate character that different from others.

![Figure 3. Variation of leaf. A. RS.136 (Java). B. BA.753A (Bali). C. DL.99 (Bali). D. PSA.215 (Bali). E. PSA.222 (Lombok). F. PSA.226 (Lombok). G. MBA.121 (Lombok). H. JQ.1143 (Lombok) (Photograph by Ni Putu Sri Asih).](image)

The generative characters, both quantitative and qualitative, showed different sizes and colors in all part of flower and peduncle (Table 3). The only similarity of those accessions is the number of inflorescence, whether it presents in several or a pair of inflorescence. Peduncle color of Bali accession shows more varied than Java and West Nusa Tenggara accession. While the limb, lower spathe, ovary and stigma color of West Nusa Tenggara accession show the most varied. The number of stigma lobe shows the same number
with Bali and West Nusa Tenggara accession (2-4 lobes), while the Java accession has different number (2-3 lobes).

Anatomical analysis
Epidermal examination on leaf anatomy of eight accessions of A. alba showed that cell wall on adaxial epidermal has anticlinal straight, angular or rounded and undulate anticlinal cell wall, whereas on abaxial, it is undulate, sinuous, straight and rounded anticlinal cell wall (Figure 4). Both abaxial and adaxial on periclinal wall are smooth. In this study, all accessions of A. alba

| No | Characters                     | Java                     | Bali                        | West Nusa Tenggara |
|----|-------------------------------|--------------------------|-----------------------------|---------------------|
| 1  | Plant height (cm)             | 129.8-132                | 114-300                     | 82.5-242            |
| 2  | Petiole length (cm)           | 83.4-96.8                | 66.9-99.2                   | 77.2-102.5          |
| 3  | Petiole color                 | Moderate yellowish green 138A | Strong yellow green C N144C | Greyish olive green A NN137A |
|    |                               |                          |                              | Moderate yellow green C 138 C |
|    |                               |                          |                              | Greyish olive green B NN 137B |
| 4  | Pattern or line on petiole    | Present                  | Present                     | Absent-present      |
| 5  | Pattern/line color            | Dark purplish grey A N187A | Greyish reddish brown B 200 | Dark purplish grey A N 186A |
|    |                               |                          |                              | Dark greyish reddish brown A 200 |
| 6  | Sheath length (cm)            | 34.9-40.3                | 26.8-65                     | 31-49               |
| 7  | Leaf shape                    | Ovate-sagittate          | Suborbicular-sagittate      | Ovate-sagittate     |
|    |                               |                          | Ovate-sagittate             | Cordate-sagittate   |
| 8  | Leaf color                    | Greyish olive green NN137A | Greyish olive green B NN137B | Greyish olive green A MM37 |
|    |                               |                          | Greyish olive green A NN137A | Greyish olive green B NN137 B |
|    |                               |                          | Greyish olive green B NN 137A | Greyish olive green A NN137A |
| 9  | Leaf edge                     | Sinuate                  | Undulate                    | Undulate            |
| 10 | Spread of posterior leaf      | Separated                | United-separated           | Separated           |
| 11 | Leaf length (cm)              | 60-68                    | 51.8-94                     | 48.5-94             |
| 12 | Leaf width (cm)               | 51.8-62.5                | 51.8-94                     | 36.6-67             |
| 13 | Apex                          | Shortly acuminate        | Shortly acuminate           | Shortly acuminate   |
|    |                               |                          | Acuminate                   | Acuminate           |
| 14 | Inner side of posterior lobe  | Obovate                  | Obovate                     | Obovate             |
|    |                               |                          | Narrowly obovate            | Narrowly obovate    |
|    |                               |                          | Lanceolate                  | Lanceolate          |
| 15 | Posterior costae diverging (°) | 135-150                 | 65-110                      | 75-135              |
| 16 | Primary lateral vein          | 7-10                     | 8-12                        | 8-14                |
| 17 | Axillary glands color         | White NN155D             | White NN155D                | White NN155D        |
|    |                               |                          | Brilliant yellow green 149 C | Strong yellow green 145 A |
| No | Characters                        | Java                       | Bali                          | West Nusa Tenggara                      |
|----|-----------------------------------|----------------------------|-------------------------------|----------------------------------------|
| 1  | Inflorescences                    | several at the centre of leaf crown, occasionally a pair | several at the centre of leaf crown, occasionally a pair | several at the centre of leaf crown, occasionally a pair |
| 2  | Peduncle length (cm)              | 21-28                      | 25-37                         | 23.5-40                                |
| 3  | Peduncle color                    | Light yellow green D144    | Moderate yellow green D139    | Strong yellow green A144               |
|    |                                   |                            | Moderate yellow green C139    | Strong yellow green A143               |
|    |                                   |                            | Moderate yellow green D137    | Light yellow green B145                |
|    |                                   |                            | Strong yellow green C143     | Strong yellow green C143               |
|    |                                   |                            | Strong yellow green C144     | Strong yellow green C144               |
|    |                                   |                            | Strong yellow green C145     | Strong yellow green C145               |
|    |                                   |                            | Brilliant yellow green C142  |                                        |
| 4  | Spathae length (cm)               | 12.7-14.6                  | 13.6-18.6                     | 10.7-17.2                              |
| 5  | Limb color                        | Brilliant yellow green C150 | Light yellow green B146       | Strong yellow green C144               |
|    |                                   |                            | Strong yellow green D1144     | Strong yellow green A145               |
|    |                                   |                            | Strong yellow green D1145     | Moderate yellow green A138             |
|    |                                   |                            | Strong yellow green C144     | Strong yellow green C144               |
|    |                                   |                            | Strong yellow green A145     | Strong yellow green B145               |
|    |                                   |                            | Strong yellow green C145     | Strong yellow green C145               |
|    |                                   |                            | Brilliant yellow green C154  |                                        |
| 6  | Lower spathe color                | Moderate yellow green B146 | Brilliant yellow green C154   | Strong yellow green A144               |
|    |                                   |                            | Brilliant yellow green C150  | Strong yellow green B144               |
|    |                                   |                            |                              | Strong yellow green C144               |
|    |                                   |                            |                              | Strong yellow green C144               |
|    |                                   |                            |                              | Strong yellow green B145               |
|    |                                   |                            |                              | Strong yellow green C150               |
|    |                                   |                            |                              | Brilliant yellow green C154            |
| 7  | Spadix length (cm)                | 9.3-11.1                   | 10.6-13                       | 7.8-11.6                               |
| 8  | Stipitate (mm)                    | 2-6                        | 1-4                           | 1-7                                    |
| 9  | Female zone length (cm)           | 2-2.4                      | 1.9-2.9                       | 1.5-2.3                                |
| 10 | Sterile interstice length (cm)    | 0.7-0.8                    | 0.6-1.4                       | 0.5-1.6                                |
| 11 | Male zone length (cm)             | 2.4-3                      | 2.3-3.4                       | 1.8-3.5                                |
| 12 | Appendix length (cm)              | 3.6-5.4                    | 5.3-6.9                       | 3.2-5.4                                |
| 13 | Ovary color                       | Strong yellow green C1144  | Light yellow green C145      | Light yellow green D150                |
|    |                                   | Strong yellow green C1144  | Strong yellow green D1144    | Light yellow green B145                |
|    |                                   | Strong yellow green C1144  | Strong yellow green D1144    | Brilliant yellow green C149            |
|    |                                   | Light green yellow C1      | Light green yellow C1        | Strong yellow green D1144              |
|    |                                   |                            |                              | Strong yellow green D1144              |
|    |                                   |                            |                              | Strong yellow C144                     |
|    |                                   |                            |                              | Strong yellow C144                     |
| 14 | Lobe number of stigma             | 2-3                        | 2-4                           | 2-4                                    |
have similar anatomical characters. *A. alba* leaf type is ampishomatic which means that stomata occur in both surfaces, but the stomatal density on adaxial is less than abaxial surface. Types of stomata on *A. alba* are anomocytic, anisocytic, paracytic and brachyparatetracytic (Figure 4). The latter stomata type was based on Dilcher (1974). Transversal section of leaf showed 1-2 layer of palisade on adaxial side of the leaf and sponge tissue arranged below the palisade (Figure 5 A). All accessions have druse shape of CaCO$_3$ crystal (Figure 5 B).

![Figure 4](image-url)

Figure 4. Leaves epidermal on *A. alba*. Anticlinal epidermal wall with undulate cell (A), angular and rounded cell (B). sinuous (C). Stomata type of brachyparatetracytic (b) also presents in *A. alba* (D). There are stomata types i.e. anomocytic (a), anisocytic (ai) and paracytic (p). Epidermal cell (e). Scale bar 50 μm.

The transversal section of *A. alba* showed that the leaf consists of cuticle, epidermal, palisade and sponge cells (Figure 5). The cuticle is situated in

| No | Characters                    | Java                        | Bali                          | West Nusa Tenggara |
|----|-------------------------------|-----------------------------|-------------------------------|--------------------|
| 15 | Stigma color                  | Pale green yellow D 2       | Light yellow green D 150      | Light greenish yellow D7 |
|    |                               | Pale green yellow D 3       | Pale greenish yellow D1       | Light greenish yellow D8 |
|    |                               |                             | Light yellow green D 150      | Light yellow green D 150 |
|    |                               |                             | Strong green yellow B 151     | Strong green yellow B 152 |
| 16 | Sterile interstice color      | Pale yellow pink D159       | Yellowish white D 158         | Pale yellow B 158 |
|    |                               |                             | Yellowish white D 158         | Yellowish white B 155 |
|    |                               |                             | Yellowish white D 158         | Yellowish white D 158 |
| 17 | Male zone color               | Pale yellow pink D159       | Yellowish white D 158         | Pale yellow B 158 |
|    |                               | Yellowish white C158        | Yellowish white D 155         | Yellowish white B 155 |
|    |                               |                             | Yellowish white C 155         | Yellowish white C 158 |
| 18 | Appendix color                | Light yellow pink A 159     | Yellowish white C 158         | Pale yellow B 158 |
|    |                               | Pale yellow A 158           | Yellowish white D 158         | Pale yellow A 158 |
|    |                               |                             | Yellowish white A 158         | Yellowish white D 155 |
|    |                               |                             | Yellowish white D 158         | Yellowish white D 158 |

Table 3. Contd.
adaxial surface, while the one layer of epidermal cell is situated in both surfaces. The sponge cell has the thickest part in leaf tissue.

Table 4 showed stomata and epidermal measurement. Stomatal density from Java is higher than from Bali and West Nusa Tenggara. The stomata are longer and wider as well as the epidermal cells are thicker in the adaxial than abaxial side. This study also showed that Bali’s accessions have longer stomata; thicker epidermis, palisade and sponge compared to accessions of other locations. But, the adaxial stomata of Java accession are the widest.

![Figure 5](image_url). The transversal section of *A. alba* leaf. A.) Bar scale 100 μm. B.) s: sponge; p: palisade; le: lower palisade; ue: upper palisade. Scale bar 50 μm.

**Discussion**

Eight accessions of *A. alba* from Java, Bali and West Nusa Tenggara were observed based on morphological and anatomical characters. Predominantly, the variations in morphological are the color of petiole, pattern of petiole, leaf, peduncle, spathe and spadix of *A. alba* (Table 2 and Table 3). Related to the present of patterns in petiole, it is divided into two variations i.e. petiole with pattern and petiole without-pattern. Petiole without-pattern only found in accession from West Nusa Tenggara. These kinds of variations have never been studied but have founded in several variations in *Alocasia longiflora* Miq.
*A. longiloba* have seven peak variations and mostly have mottled petiole, but the petiole of watsoniana variation is not or faintly mottled. This immottled petiole sometime also founded in lowii variation. The cause of variations are still not understood (Hay 1998).

According to the color of petiole pattern, there are four variations of color. These variations of colors have never been reported in *Alocasia* genus, but has reported in *Colocasia esculenta* (Maretta et al. 2020) and other family, Begoniaceae (Efendi et al. 2020). The differences of colors might be as a response to different light intensities that are obtained by the plant (Zhang et al. 2018).

This study also found some variations in leaf shapes, sizes of petioles, leaves, peduncles, spathes and each zone of spadix. This phenotypic variation within species is the result of the interaction of environmental and genetic factors that was gradually inherited to the offspring (Ramsey et al. 1994; Gonzalez et al. 2012; Albarrán-Lara et al. 2018; Li et al. 2018; Alcántara-ayala et al. 2020; Ren et al. 2020). The leaf size and shape indicated the diversity of leaf morphological phenotypes (Ren et al. 2020).

Meanwhile, the epidermal character of the eight accessions of *A. alba* showed similarities, especially in qualitative parameters. The similarity of cell form in the adaxial and abaxial surface is commonly found in plants, even though it is also found the different forms between those two surfaces (Cutler et al. 2007). The leaf anatomy of *A. alba* had been observed by Erlinawati & Tihurua (2013). The observed characters were epidermal cell shape, anticlinal wall, distribution of stomata, and the present of trichome. Erlinawati & Tihurua (2013) mentioned that the anticlinal wall of *A. alba* was straight but, the eight accessions of *A. alba* on this study, showed that it is also found the undulate and sinuous anticlinal cell wall. These differences can give new information about the range or variation of *A. alba* epidermal characters.

The stomata of *A. alba* are found in adaxial and abaxial, and it has four types of stomata *i.e.* anomocytic, anisocytic, paracytic, and brachyparatetra-cytic (Figure 4). Some studies about the stomata type of Araceae have been conducted in *A. cucullata, A. macrorrhiza* and *A. plumbea* (Suratman et al. 2016; Arogundade & Adedeji 2019), some Araceae species in Bombay and Maharashtra (Vaidya 2016b), and some species of *Alocasia, Colocasia* and *Remusatia* in Indonesia (Erlinawati & Tihurua 2013). Those three studies found one type of stomata in each species, but other research discovered two types of stomata (Sookchaloem et al. 2016; Vaidya 2016a). Those agreed to Cutler et al. (2007) which stated that although most species only have one type, but some species can have several types of stomata.

The other stomata character, density of stomata, showed that the highest density belongs to Java accession. The fact that the stomata on the abaxial side are denser than adaxial side has also confirmed by several research in *Alocasia* (Arogundade & Adedeji 2019; Suratman et al. 2016). Kondo et al. (2010) mentioned that environment condition is one of factor that affects the
density of stomata in plants. The dependency of this character to the environment condition can be used as indicator of transpiration and photosynthesis rate; also on absorption of water and mineral by the plant (Suratman et al. 2016; Rindyastuti & Hapsari, 2017). The quantitative data such as stomata length and width, epidermal thickness, palisade thickness, and sponge thickness and leaf thickness showed the variation amongst examined accession from three locations. Commonly, the Bali accession has the highest of all characters measurement and it might be caused by the adaptation of plant to the environment factors (Suratman et al. 2016).

The fact that the character variations of A. alba accessions from different locations, Java, Bali and West Nusa Tenggara, which planted in Bali Botanic Garden Conservatory that relatively has same environment condition might be caused by the genetic factor that the plant inherited from the parental and adaptation to the different physical condition for long time. Research about plant variation in different environments is important to understand the genetic diversity, genetic breeding and basis of conservation biology (Li et al. 2018) completed with the evolutionary processes that might promote speciation and maintain diversity (Alcántara-ayala et al. 2020). Furthermore, for biology conservation, plant variation research can give more specific information about the species that has to be conserved, especially the wild species to prevent genetic diversity loss (Santos et al. 2012).

CONCLUSION
This study showed that there are some variations within species of A. alba from different locations based on their morphological and anatomical characteristics. These variations can be caused by genetic factors as a result from plant adaptation to different environments. Therefore, to prove the genetic factors on these variations, more data of morphology, anatomy and molecular are needed to enrich the information of A. alba.

AUTHORS CONTRIBUTION
N.P.S.A did the morphological observation, analysis and write manuscript. E.H. did the morphological observation, stomatal density measurement and write manuscript. E.F.T. did the anatomical preparation, observation, analysis and write manuscript.

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CONFLICT OF INTEREST
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