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Research Article

Morphological and Anatomical Variations among *Alocasia alba* Schott Accessions in Bali Botanic Garden

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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.

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based on herbarium tracking and field observation, it is estimated about 36 *Alocasia* species origin from Indonesia (unpublished data).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.

Table 1. Collection and access number of eight accessions *A. alba* in Bali Botanic Garden.

No	Collection/Access Number	Location	Altitude (m asl)
1	PSA.222/E2014120016	Sendang Gile Waterfall, North Lombok, WNT	471
2	JQ.1143/E2017080061	Benang Kelambu Waterfall, Central Lombok, WNT	577
3	MBA.121/E2016050041	Rinjani Mountain National Park, East Lombok, WNT	911
4	PSA.226/E2014120020	Benang Kelambu Waterfall, Central Lombok, WNT	537
5	BA.753A/E20110952	Munduk Pengubengan, Karangasem, Bali	1060
6	RS.136/E2014020001	Merapi Mountain National Park, Sleman, Yogyakarta	1004
7	PSA.215/E2014120009	Seraya Mountain, Karangasem, Bali	788
8	DL.99/E2015110012	Grojogan River, Jembrana, Bali	196

Figure 2. Distribution of *A. alba* in Java, Bali and West Nusa Tenggara. (Google earth and modified by Ni Putu Sri Asih (unpublished data)).

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 occured 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.

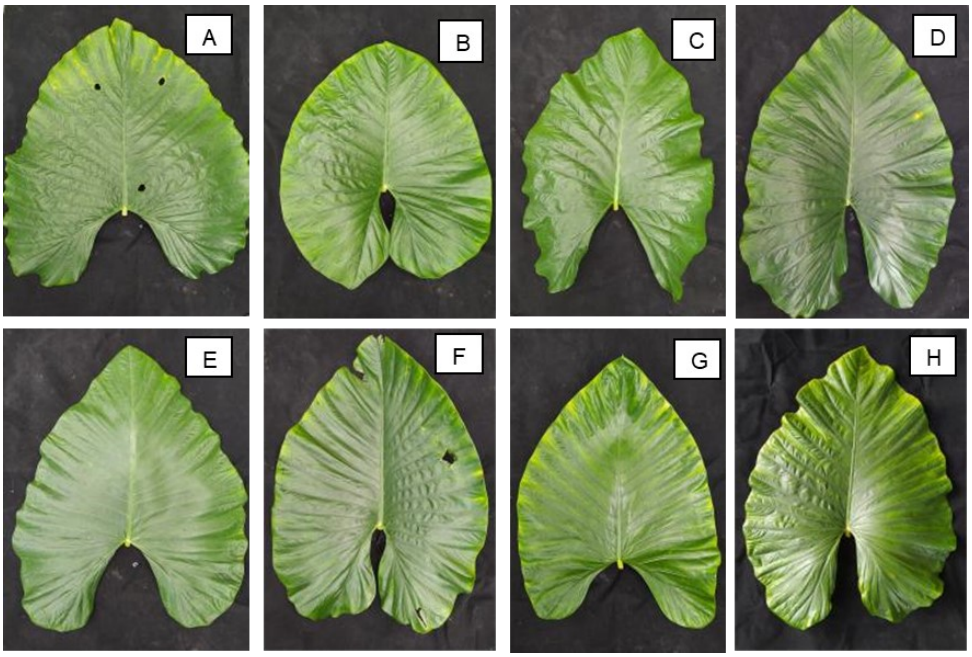

Figure 3. Variation of leaf. A. RS.136 (Java). B. BA.753A (Bali). C. DL.99 (Bali). D. PSA.215 (Bali). E. PSA.222 (Lombok). F. PSA.226 (Lombok). G. MBA.121 (Lombok). H. JQ.1143 (Lombok) (Photograph by Ni Putu Sri Asih).

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

Table 2. Vegetative characters of *A. alba* in Bali Botanic Garden based on collections origined Java, Bali and West Nusa Tenggara.

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 yellow-ish green 138A	Strong yellow green C N144C Greyish olive green A NN137A Greyish olive green B NN 137B	Strong yellow green 145A Moderate yellow green C 138 C Moderate yellow green B 146A Greyish olive green A NN137A
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 greyish reddish brown A 200	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 Cordate-sagittate	Ovate-sagittate Cordate-sagittate
8	Leaf color	Greyish olive green NN137A	Greyish olive green B NN137B Greyish olive green A NN137A Greyish olive green B NN 137A	Greyish olive green A MM37 Greyish olive green B NN137 B Greyish olive green A NN137A
9	Leaf edge	Sinuate	Undulate	Undulate Slightly 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 acumin-ate	Shortly acuminate Acuminate	Shortly acuminate Acuminate
14	Inner side of posterior lobe	Obovate	Obovate Narrowly obovate Lanceolate	Obovate Narrowly obovate
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 Brilliant yellow green 149 C	White NN155D Strong yellow green 145 A

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*

Table 3. Generative characters of *A. alba* in Bali Botanic Garden based on collections origined Java, Bali, and West Nusa Tenggara.

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 D 139 Moderate yellow green C 139 Moderate yellow green D 137 Strong yellow green C 143 Strong yellow green C 144 Strong yellow green C 145 Brilliant yellow green C 142	Strong yellow green A 144 Strong yellow green A 143 Light yellow green B 145 Strong yellow green C 143 Strong yellow green C 144
4	Spathe length (cm)	12.7-14.6	13.6-18.6	10.7-17.2
5	Limb color	Brilliant yellow green C 150	Light yellow green B 146 Strong yellow green D N144 Strong yellow green D N145	Strong yellow green C 144 Strong yellow green A 145 Moderate yellow green A 138 Strong yellow green A 144 Light yellow green B 145 Light yellow green D 154
6	Lower spathe color	Moderate yellow green B 146	Brilliant yellow green C 154 Brilliant yellow green C 150	Strong yellow green A 144 Strong yellow green B 144 Strong yellow green C 144 Strong yellow green A 145 Strong yellow green B 145 Brilliant yellow green C 150 Brilliant yellow green C 154
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 C N144 Strong yellow green C N144	Light yellow green C 145 Strong yellow green D N144 Light green yellow C1	Light yellow green D150 Light yellow green B 145 Brilliant yellow green C 149 Strong yellow green D N144 Strong yellow C 144 Strong yellow Green A 145
14	Lobe number of stigma	2-3	2-4	2-4

Table 3. Contd.

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

have similar of 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).

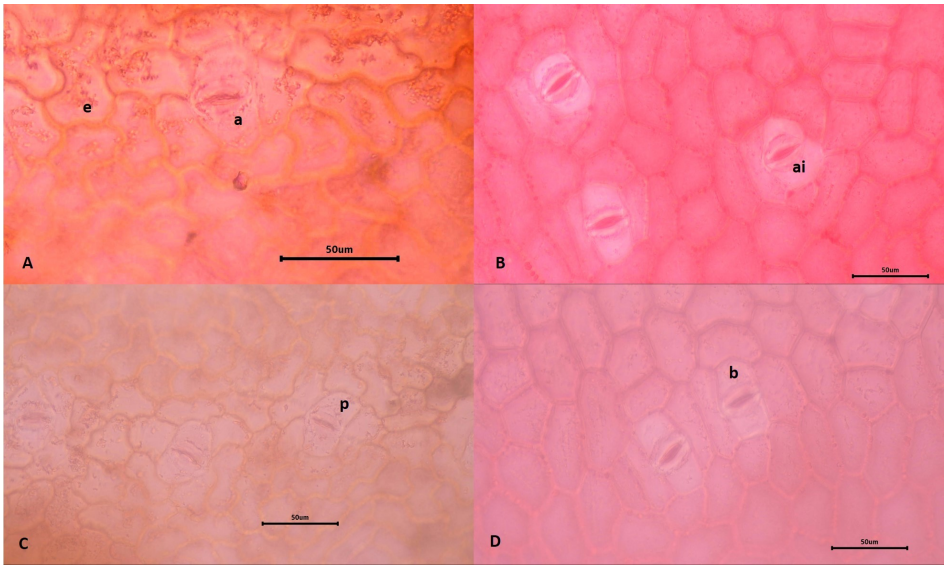

Figure 4. Leaves epidermal on *A. alba*. Anticlinal epidermal wall with undulate cell (A). angular and rounded cell (B). sinuous (C). Stomata type of brachyparatetracytic (b) also presents in *A. alba* (D). There are stomata types *i.e.* anomocytic (a), anisocytic (ai) and paracytic (p). Epidermal cell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

Table 4. Anatomical characters of *A. alba* in Bali Botanic Garden based on collections origined Java, Bali, and West Nusa Tenggara.

Parameter		Collection Origin		
		Java	Bali	West Nusa Tenggara
Density of stomata (number of stomata/mm²)	Adaxial	54.78 ± 2.88	41.7 ± 6.33	25.2 ± 7.26
	Abaxial	106.11 ± 8.86	80.95 ± 16.93	87.27 ± 10.43
Length of stomata (µm)	Adaxial	32.31 ± 2.20	34.81 ± 3.27	32.8 ± 2.00
	Abaxial	29.52 ± 2.13	33.05 ± 2.79	31.53 ± 1.76
Width of stomata (µm)	Adaxial	28.37 ± 2.67	25.71 ± 2.97	24.93 ± 1.86
	Abaxial	23.62 ± 2.28	25.99 ± 2.46	23.91 ± 2.55
Epidermal Thickness (µm)	Adaxial	22.63 ± 2.99	23.53 ± 4.14	20.05 ± 2.98
	Abaxial	19.35 ± 3.97	17.51 ± 3.28	16.73 ± 3.78
Palisade Thickness (µm)		49.53 ± 10.31	52.52 ± 12.77	49.61 ± 10.27
Sponge Thickness (µm)		161.70 ± 25.59	193.60 ± 26.35	156.17 ± 20.29
Leaves Thickness (µm)		253.22 ± 23.40	287.62 ± 30.64	242.56 ± 25.66

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.

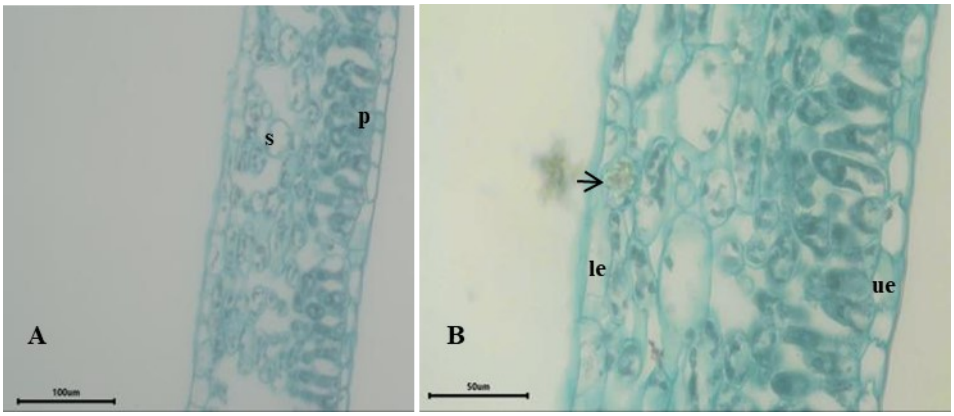

Figure 5. The transversal section of *A. alba* leaf. A.) Bar scale 100 µm. B.) s: sponge; p: palisade; le: lower palisade; ue: upper palisade. Scale bar 50 µm.

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 longiloba* 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 brachyparatetracytic (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

The authors state that there is no any conflict of interest regarding the research or the research funding.

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