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Phenotypic and Estimated Genetic Variability in Endemic *Diospyros celebica* Bakh. and Widely-spread *D. blancoi* A.Dc. Cultivated at Purwodadi Botanic Garden, Indonesia

Ilham Kurnia Abywijaya[1], Ridesti Rindyastuti[2,3], Fira Fitria Jihans[3], Anjar Tri Wibowo[4*]

[1]*Research Center for Ecology and Ethnobiology, National Research and Innovation Agency (BRIN), Cibinong 16911, Indonesia*
[2]*Research Center for Applied Botany, National Research and Innovation Agency (BRIN), Cibinong 16911, Indonesia*
[3]*Department of Biology, Faculty of Mathematics and Natural Science, Malang State University, Malang 65145, Indonesia*
[4]*Faculty of Science and Technology, Universitas Airlangga, Surabaya 60115, Indonesia*

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ABSTRACT

The endemic *Diospyros celebica* and widely spread *D. blancoi* are two valuable species of *Diospyros* (the genus of persimmons and ebonies) that have been conserved at botanic gardens in Indonesia. Understanding the biological contrasts between endemic and widely spread species is essential for plant conservation. This study aims to compare the phenotypic and estimated genetic variability between the endemic *D. celebica* and the widely spread *D. blancoi* to support their conservation. Fifty-three morphological characters from fifteen selected living plant specimens at Purwodadi Botanic Garden were measured directly or using variable encoding methods. Unpaired two-samples Wilcoxon test and hierarchical cluster analysis were used to compare morphological variations between both species. Further, genetic variabilities were estimated from encoded morphological characters using GenAlEx 6.503 software. The two *Diospyros*' morphological characters were significantly different, except for the lamina length, pairs of leaf vein, number of flowers in each inflorescence, and the fruit sulci presence. The cluster analysis successfully distinguished *D. celebica* from *D. blancoi* according to thirteen comparable morphological characters. The endemic *D. celebica* has lower genetic variability (female/bisexual %P = 86.21%, He = 0.259, uHe = 0.277; male P = 54.29%, He = 0.195, uHe = 0.234) than the widely spread *D. blancoi* (female/bisexual %P = 86.76, He = 0.311, uHe = 0.355). Due to the importance of genetic diversity in plant conservation, both species' population size needs to be maintained, if not increased, to preserve the existing phenotypic and genetic variabilities within the cultivated populations at the botanic garden.

1. Introduction

Diospyros (family: Ebenaceae) is an ecologically and economically important group of flowering plants, covering many species of ebonies and persimmons. Its members comprise more than 500 species worldwide, with most species found across the Indo-Pacific region (Wallnöfer 2001; Wanda *et al.* 2021). Plant species belonging to this genus are mostly dioecious with separated male and female plants. They have simple alternate leaves, with axillary cyme inflorescences or pistillate solitary flowers in white or yellow colors, and berry fruits with persistent calyx (Bakhuizen van den Brink 1938; Wallnöfer 2004).

Some *Diospyros* species have been widely cultivated for their flavorsome fruits and various commercial uses. This delicious fruit-producing group of *Diospyros* is commonly known as "persimmons". One example of persimmons in Indonesia is the velvet persimmon (*Diospyros blancoi* A.Dc.), which produces tasty, soft, and creamy velvet fruits (Hung *et al.* 2016; Nasution and Hadiati 2020). Some other *Diospyros* have been favored and overexploited due to their high quality of black colored hardwood. These species are commonly known as "ebonies" and are also known as kayu

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* Corresponding Author
E-mail Address: anjar.tri@fst.unair.ac.id

hitam in Indonesia, which means black wood. One example of ebonies from Indonesia is the renowned Sulawesi ebony (*Diospyros celebica* Bakh.) that is endemic to Sulawesi Island. It has been classified as a vulnerable species under the IUCN Red List category (WCMC 1998). Due to their economic and ecological importance, *Diospyros* conservation through botanic gardens is crucial to facilitate intensive studies on their biology and prospective sustainable uses. Such studies on *Diospyros* have been continuously carried out at the Indonesian Botanic Gardens to support the conservation of this genus (Rindyastuti 2021; Rindyastuti *et al.* 2021; Wanda *et al.* 2021).

One of the botanic gardens designated for *ex-situ* plant conservation in Indonesia is Purwodadi Botanic Garden. This botanic garden has been focusing on the conservation of plants from lowland habitats for more than sixty years. No less than 2020 plant species have been collected and cultivated in this botanic garden, including 28 species of *Diospyros* from Indonesia (Rindyastuti 2021).

For *ex-situ* plant conservation through botanic gardens, maintaining genetic diversity within the cultivated plant population is pivotal (Edwards and Jackson 2019; Kovács *et al.* 2021) since it influences the species' fitness and survival (Ratnam *et al.* 2014). However, studies on plant genetic diversity are still very limited due to the technical difficulties and the high cost of such studies, especially in developing countries (Zegeye 2016). Alternatively, phenotypic characterization has been used to estimate the level of genetic variation in plant populations. Several previous studies used highly heritable traits, such as fruit and seed morphological characters and adaptive performance, to estimate genetic variation in rare and endemic trees (Hamilton *et al.* 2017; di Santo *et al.* 2021), perennial herbs (Weber and Kolb 2014; Yoko *et al.* 2020), and crop plants (Zarei *et al.* 2020; Khadivi *et al.* 2020; Zhang *et al.* 2022).

Despite the growing interest in utilizing phenotypic traits to estimate genetic variation in plants, to date, most studies conducted quantitative morphological analyses only to accompany their genetic analyses (see Jeong *et al.* 2019; Darkwa *et al.* 2020 for crop plants and Rindyastuti *et al.* 2021; Qiao *et al.* 2022 for woody species). Some biologists argue that estimating genetic diversity through morphological variation still has several limitations (Jain *et al.* 2017) since morphological variation appears because of additive genetic variation and environments. However, such an issue can still be addressed by planting species or populations from varying sources under the same controlled conditions, namely common garden experiments (Schwinning *et al.* 2022). These experiments can minimize the effect of environments and allow the assumption of the pure effect of plants' genetics on their morphological expressions.

Most living plant specimens in botanic gardens have been collected from various geographic locations and cultivated in a relatively similar environment with equally intensive care. Thus, cultivated plant populations in botanic gardens can be assumed to partially imitate common garden experiments. Using this assumption, the present study utilized morphological variation to estimate the genetic diversity in the cultivated plant populations in Purwodadi Botanic Gardens. Two model species chosen for this study were an endemic wood-producing *Diospyros celebica* (Sulawesi ebony) and a widely spread fruit-producing *Diospyros blancoi* (velvet persimmon). Overall, this research aimed to 1) investigate and compare phenotypic variability between an endemic and a widespread tree species, *D. celebica* and *D. blancoi,* respectively, and 2) employ phenotypic characterization methods to estimate genetic variability in the two *Diospyros* populations at the garden. Information regarding phenotypic variability and estimated genetic variability collected in this study can support *ex-situ* conservation of threatened *D. celebica* and the cultivation of commonly favored *D. blancoi*. It can also be used as a basis for larger studies in their natural habitats.

2. Materials and Methods

2.1. Materials

Materials used in this study are fifteen living plant specimens of an endemic *Diospyros celebica* Bakh. and a widely spread *D. blancoi* A. DC. (Ebenaceae) cultivated at Purwodadi Botanic Garden, East Java, Indonesia. Plant materials of *D. celebica* were originally collected from wild populations in Sulawesi, Indonesia, while *D. blancoi* were originally collected from three different populations throughout Java, Indonesia. In order to maintain consistency in data collection, only healthy specimens and plant parts were observed. All living specimens with any sign of abnormal and distorted growth and pest and disease manifestations were excluded from the study.

2.2. Data Collection

2.2.1. Morphological Observation

Morphological data were collected during flowering and fruiting periods of *Diospyros* in Purwodadi Botanic Garden from May to September 2019. As many as 53 morphological characters were observed from all the selected living plant specimens. Any characters with no distinguishable feature between species were omitted from the study. Following sorting, 45 distinguishable characters remained to be used in this study (see Table 1).

2.2.2. Quantification of Categoric Morphological Data

Categorical data in plant morphological characters were quantified to operate some numerical analyses (Legendre and Legendre 2012). In this study, parameter quantification was performed using the ordinal encoding methods, similar to those used in Lestari *et al.* (2021).

2.3. Data Analysis

2.3.1. Variation in Morphological Characteristics

To compare morphological characters and analyze how significant the morphological differences between *Diospyros celebica* and *D. blancoi* are, non-parametric statistics of the Unpaired two-sample Wilcoxon test was used to address unequal sample size and non-normality in the quantified morphological data (Legendre and Legendre 2012). Additional analysis to group plant specimens by their morphological similarities was performed using the agglomerative hierarchical cluster analysis with Unweighted Pair Group Method of Arithmetic Averaging (UPGMA) linkage and Euclidian distance measures of standardized quantified morphological data. All of the analyses were performed using R 4.2.1 (R Core Team 2021) with heatmap hierarchical clusters visualized using the "ComplexHeatmap" R package (Gu *et al.* 2016).

2.3.2. Polymorphism and Heterozygosity

Plant morphological characteristics were classified into several key characters to distinguish plant specimens into groups at the intraspecific level (Rindyastuti *et al.* 2021). These key characters were treated equally as "locus" in common molecular analyses and transformed into a binary data matrix using a binary encoding method. Percent of polymorphic characters (%P), Shannon information index (I), and Heterozygosity value (He) based on Nei (1973) were calculated using GenAlEx 6.5 (Peakall and Smouse 2012). These measures of polymorphism and heterozygosity were used to estimate genetic diversity in the studied plant populations.

3. Results

3.1. Variation in Morphological Characters

Forty-five distinct morphological characters were observed across *Diospyros celebica* and *D. blancoi* living plant specimens cultivated in the Purwodadi Botanic Garden (Table 1). The observed variations varied substantially among plant parts and variables and between species. Higher qualitative variations were observed in the color and density of petiole trichomes, followed by the color of leaf trichomes and the shape of fruits. Several key characteristics, such as lanceolate leaves with acute apices and cuneate bases, globose- and ovoid-shaped densely hairy fruits, and yellowish flowers, were observed

Table 1. Forty-five distinct morphological variables and their respective number of variations were observed in both *Diospyros celebica* and *D. blancoi* (♀: female/bisexual plants, ⚥: male plants) cultivated at Purwodadi Botanic Garden

Morphological variable	Variable scale	Possible quantified morphological characters	*D. celebica*			*D. blancoi*
			⚥	♂	All	⚥
Bark color	bright to dark	(1)Brown, (2)Greyish-brown, (3)Dark brown, (4) Black	2	1	2	3
Calyx length	in centimetres	–	0	4	4	0
Calyx width	in centimetres	–	0	3	3	0
Corolla color	bright to dark	(1)Greyish-cream, (2)Cream, (3)Pinkish-cream	0	3	3	0
Corolla lobe length	in centimetres	–	6	4	6	0
Corolla lobe width	in centimetres	–	7	3	8	0
Corolla tube length	in centimetres	–	0	3	3	0
Corolla lobe length	bright to dark	(1)Golden-brown, (2)Blackish-brown, (2)Black	0	3	3	0
Corolla lobe width	count	–	0	3	3	5
Corolla tube length	in centimetres	–	0	3	3	0

Table 1. Continued

Morphological variable	Variable scale	Possible quantified morphological characters	*D. celebica*			*D. blancoi*
			⚥	♂	All	⚥
Exocarp texture	smooth to wavy	(1)Smooth, (2)Moderately wavy, (3)Wavy	3	0	3	0
Flush color	bright to dark	(1)Silverish-green, (2)Creamy-green, (3)Green	0	0	0	2
Fruit diameter	in centimetres	–	35	0	35	12
Fruit flesh texture	soft to hard	(1)Mushy, (2)Tender, (3)Fairly tough, (4)Hard	0	0	0	2
Fruit length	in centimetres	–	36	0	36	14
Fruit scent	bad to good	(1)Strong-decayed, (2)Fusty, (3)Odor-less, (4) Creamy, (5)Sweet	0	0	0	2
Fruit shape	elongated to flat-tened	(1)Ellipsoid, (2)Ovoid, (3)Rounded, (4)Globose, (5)Oblate	3	0	3	3
Fruit sulci appearance	absent to distinct	(0)Absent, (1)Fairly noticeable, (2)Distinct	3	0	3	2
Fruit taste	bad to good	(1)Bad, (2)Bland, (3)Fairly good, (4)Delicious	0	0	0	3
Mature fruit color	bright to dark	(1)Yellowish-green, (2)Green, (3)Dark green, (4) Yellowish-red	0	0	0	4
Mature fruit trichome color	bright to dark	(1)Silverish-green, (2)Orange, (3)Red-dish-orange, (4)Brown, (5)Blackish-brown	0	0	0	3
Mature fruit trichome density	absent to dense	(0)Absent, (1)Sparse, (2)Moderate, (3)Dense, (4) Extremely dense	0	0	0	2
Peduncle length	in centimetres	–	6	0	6	8
Young fruit color	bright to dark	(1)Light green, (2)Green, (3)Dark green	1	0	1	2
Young fruit trichome color	bright to dark	(1)Silverish-green, (2)Orange, (3)Red-dish-orange, (4)Brown, (5)Blackish-brown	3	0	3	1
Young fruit trichome density	absent to dense	(0)Absent, (1)Sparse, (2)Moderate, (3)Dense, (4) Extremely dense	3	0	3	2
Lamina abaxial trichome color	bright to dark	(1)Golden-yellow, (2)Silverish-brown, (3) Brown, (4)Blackish-brown, (5)Black	3	3	3	3
Lamina abaxial trichome density	absent to dense	(0)Absent, (1)Sparse, (2)Moderate, (3)Dense	3	3	3	0
Lamina adaxial color	bright to dark	(1)Light green, (2)Yellowish-green, (3)Green, (4) Dark green	4	2	4	2
Lamina apex shape	pointy to blunt	(1)Acuminate, (2)Acute	2	2	2	2
Lamina base shape	pointy to blunt to notching inward	(1)Cuneate, (2)Rounded, (3)Truncate, (4)Sub-cordate, (5)Cordate	2	1	2	4
Leaf glands appearance	absent to distinct	(0)Absent, (1)Unclear, (2)Distinct	0	0	0	3
Leaf glands pairs	count	–	0	0	0	9
Lamina length	in centimetres	–	60	28	77	27
Lamina shape	slim to broad	(1)Lanceolate, (2)Elliptic-lanceolate, (3)Elliptic, (4)Oblong-elliptic, (5)Oblong, (6)Ovate	2	2	2	4
Lamina texture	thin to thick	(1)Membranaceous, (2)Chartaceous, (3)Sub-coriaceous, (4)Coriaceous	3	2	3	2
Lamina width	in centimetres	–	28	18	31	20
Margin indentation	straight to rippled	(1)Entire, (2)Sub-undulate, (3)Undulate	2	2	2	1
Petiole length	in centimetres	–	5	7	8	6
Petiole trichome color	bright to dark	(1)Grey, (2)Silverish-brown, (3)Golden-brown, (4)Brown, (5)Blackish-brown	5	5	5	3
Petiole trichome density	absent to dense	(0)Absent, (1)Sparse, (2)Moderate, (3)Dense	4	4	4	0
Secondary vein texture	protruding to sunken	(1)Salient, (2)Prominent, (3)Promi-nulous, (4) Plane, (5)Impressed	0	0	0	2
Vein pairs	count	–	13	9	13	11
Vein trichome color	bright to dark	(1)Blackish brown, (2)Black	0	0	0	2
Vein trichome density	absent to dense	(0)Absent, (1)Sparse, (2)Moderate, (3)Dense	3	3	3	2

in *D. celebica* (Figure 1). Meanwhile, oblongelliptic leaves with acute apices rounded to cuneate bases and hairy fruits in globose to oblate shapes were also found in *D. blancoi* (Figure 2).

3.2. Morphological Comparisons

A comparison of the morphological characteristics of *Diospyros celebica* and *D. blancoi* highlighted several morphological contrasts between the two species (Figure 3). These two *Diospyros* were significantly different in all characteristics except the lamina length, pairs of leaf veins, number of flowers in each inflorescence, and fruit sulci appearance. *Diospyros celebica* was significantly brighter, more elongated in the fruits and leaf shape, and shorter in peduncles and petioles length. Meanwhile, *D. blancoi*

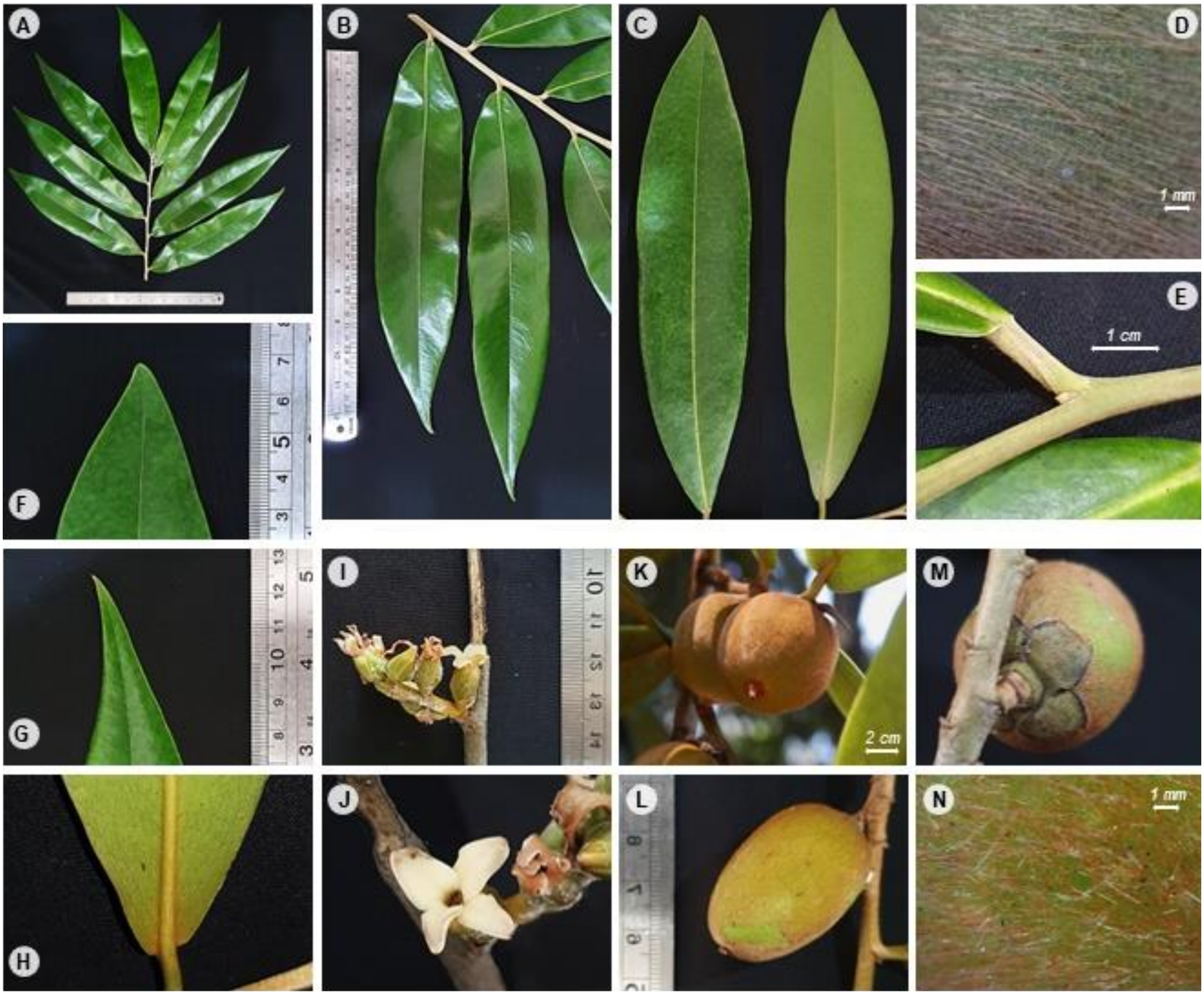

Figure 1. Distinct morphological characters in *Diospyros celebica* exhibiting: lanceolate lamina shape (A, B) with dark green adaxial color (C—left) and yellowish green abaxial color (C—right); leaf abaxial densely hairy with silverish-brown color (D); petiole is around one centimeter in length I; acute (F) or acuminate (G) leaf apex and cuneate leaf base (H); flower is almost one centimeter in size (I) with creamy corolla (H); fruits in rounded (K) or ovoid shape (L, M) covered in dense to moderate brown hairs (K, L, M, N)

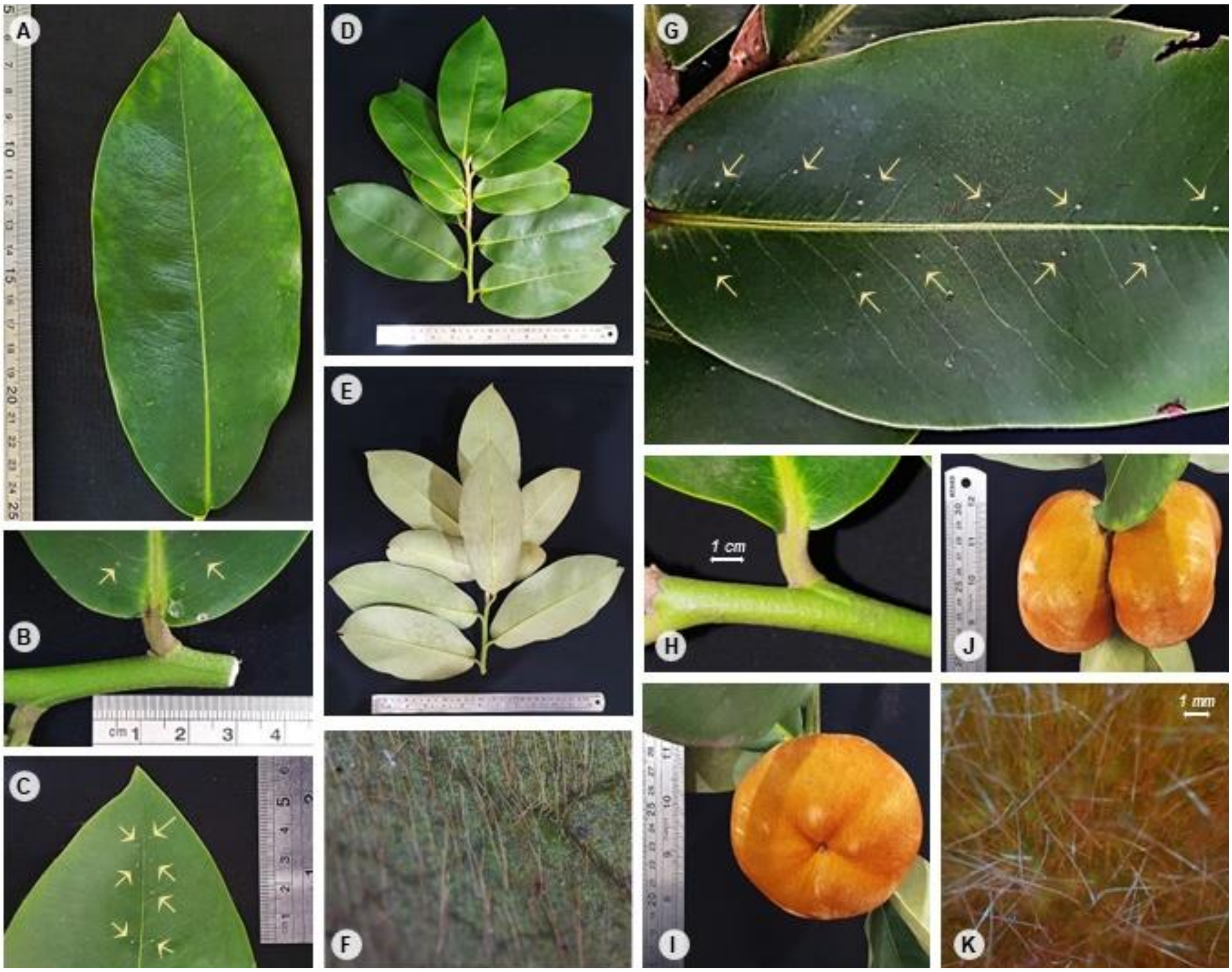

Figure 2. Distinct morphological characters in *Diospyros blancoi* exhibiting: oblong-elliptic lamina shape (A) with rounded leaf base (B) and acute leaf apex (C); leaf adaxial in green color (D) and abaxial in light-green (E) visible glands on leaf surface (B, C, G— arrows); petiole 1–1.5 centimeter in length (H); fruit shape globose to oblate, almost ten centimeter in diameter (I, J), densely hairy in brown and reddish-orange color (K)

was significantly brighter in leaf and bark color than *D. celebica* (see also Table 1 for the scale of qualitative morphological characters).

The hierarchical cluster analysis successfully discriminated between *D. celebica* and *D. blancoi* according to the thirteen comparable morphological characters (Figure 4). Furthermore, the analysis could also group all male specimens of *D. celebica*, although no distinguishable morphology was visible. This result suggests that vegetative morphological characters alone can differentiate one species from another but are still visually insufficient to distinguish between male and female plants. The use of only thirteen vegetative characters in all specimens was due to the disproportionate appearance of sex related organs across all specimens. The absence of data related to female flowers and fruits in male *Diospyros* specimens, and contrariwise, the absence of male flower data in female and bisexual specimens, prevents many of the observed characters from being incorporated into the cluster analysis.

According to the hierarchical clustering, *D. blancoi* were able to be distinguished from *D. celebica* by their darker lamina abaxial trichome color, blunter lamina apex, broader and longer lamina, and denser leaf vein trichome (Figure 4, with Table 1 assistance). On the other hand, most *D. celebica* had darker bark and lamina adaxial color, thicker lamina, and more rippled in their leaf margin indentation. The hierarchical clustering also highlighted the

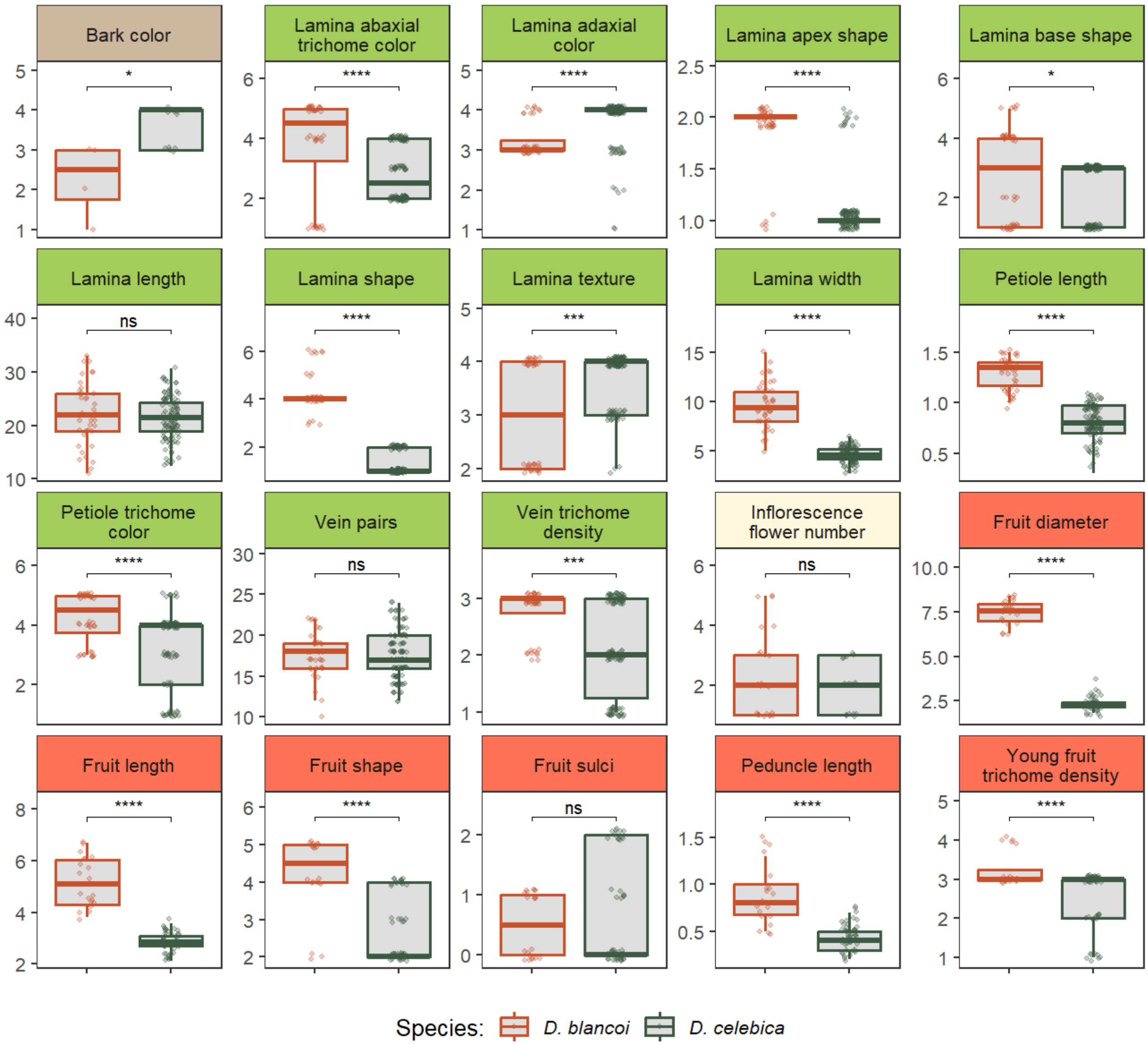

Figure 3. A comparison of twenty morphological characters between *Diospyros celebica* and *D. blancoi* was presented in faceted boxplots with points representing observed values. Background colors in plot labels indicate different plant parts being compared, with brown = bark, green = leaf, cream = flower, and red = fruit. Symbols above two boxplots' linkage signify Unpaired two-samples Wilcoxon test results at $\alpha = 0.05$: ns = not significant, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, and **** p-value < 0.0001

importance of leaf shape, apex, width, and petiole length in distinguishing between the two species.

3.3. Polymorphism and Heterozygosity

The four calculated measures of genetic diversity revealed high genetic variabilities in the living specimens of both *D. celebica* and *D. blancoi* cultivated in Purwodadi Botanic Garden (Table 2). Compared to the endemic *D. celebica*, the widely spread *D. blancoi* possessed a slightly higher frequency of polymorphic characters (%P) and population genetic information (I) but substantially higher genetic diversity (He & uHe). The male population of *D. celebica* exhibited a substantially lower polymorphism (%P), genetic information (I), and heterozygosity (He) compared to the female/bisexual population of both *D. celebica* and *D. blancoi*. However, based on the heterozygosity measure that was unbiased by population size (uHe), the male population of *D. celebica* only had a slightly lower genetic diversity than its female/bisexual counterpart.

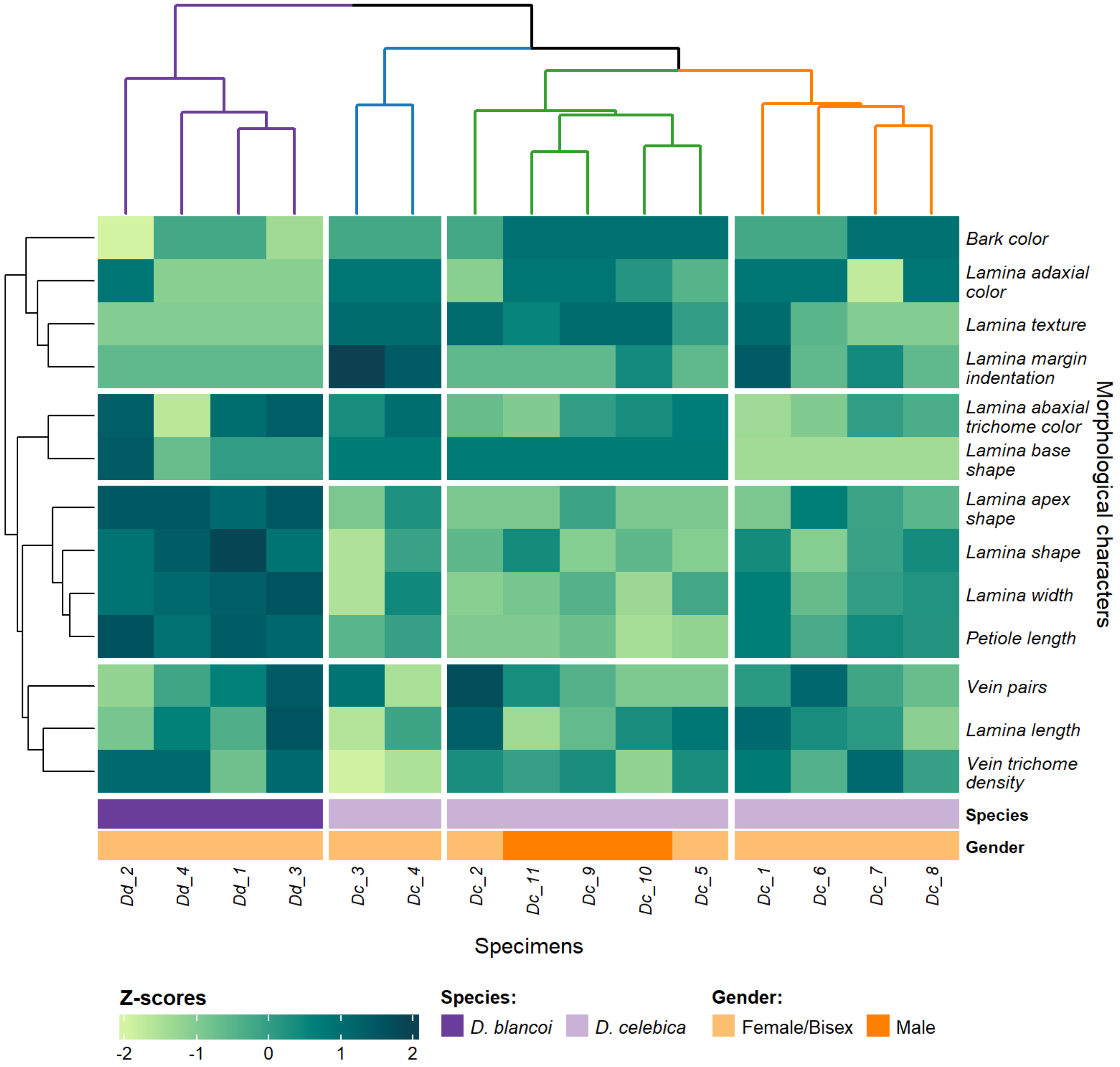

Figure 4. Heatmap hierarchical clustering of fifteen living specimens of ebony, the endemic *Diospyros celebica* and widely spread *D. blancoi*, cultivated at Purwodadi Botanic Gardens. Euclidian distance measure and UPGMA clustering methods were used to develop the heatmaps from standardized quantified morphological characters (Z-score). Rows represent morphological characters, while columns represent specimens (with some arbitrary codes that represent the specimens at the bottom of the heatmap). A four-colored dendrogram at the top signifies the four distinct groups of all *Diospyros* specimens, while purple and orange annotation marks at the bottom indicate the specimen's species identity and gender, respectively

Table 2. The calculated measures of genetic variability in the living specimens of endemic *Diospyros celebica* and widespread *D. blancoi* cultivated in Purwodadi Botanic Garden

Species	Gender	Percent of polymorphism (%P)	Shannon information index (I)	Expected heterozygosity (He)	Unbiased expected heterozygosity (uHe)
Diospyros celebica Bakh.	Female/bisexual	86.21	0.404	0.259	0.277
Diospyros celebica Bakh.	Male	54.29	0.294	0.195	0.234
Diospyros blancoi A.Dc.	Female/bisexual	86.76	0.467	0.311	0.355

4. Discussion

4.1. Comparison of morphological characters

Diospyros celebica was reported to have linear-elliptical-shaped leaves with 12–35 cm × 2.5–7 cm in size, while *D. blancoi* was reported to have oblong to elliptical-oblong leaves in the size of 8–30 cm × 2.5–12 cm (Bakhuizen van den Brink 1938; Lemmens *et al.* 1995). Meanwhile, our large morphological dataset of living *Diospyros* specimens cultivated *ex-situ* at Purwodadi Botanic Garden recorded the leaf size range of 12.5–30.7 cm × 2.8–6.5 cm in *D. celebica* and 11–33 cm × 5–15 cm in *D. blancoi*. Our observation results conformed with the literature on the leaf size of both species, although several leaves in *D. blancoi* were recorded as slightly bigger than in the literature. Some other captured variations in leaf morphology that slightly differed from the literature were the lanceolate leaf shape, with cuneate and truncate bases in *D. celebica*, as well as elliptic and ovate leaf shapes, with cuneate and cordate bases, and acuminate apices in *D. blancoi*. Despite these small anomalies, the relative comparison between the two species was still like the literature that described slenderer shapes and more pointed tips in *D. celebica* leaves compared to the leave.

Some other key morphological characters that were reported in the literature were the ovoid-shaped fruits with a diameter of 3.5–5 cm in *D. blancoi* and globose to oblate-shaped fruits with a diameter of 7–10 cm in *D. blancoi* (Bakhuizen van den Brink 1938; Lemmens *et al.* 1995). Meanwhile, our observation recorded not only ovoid but also rounded and globose-shaped fruits in *D. celebica* with 1.68–3.66 cm in diameter and not only globose and oblate but also ovoid-shaped fruits in *D. blancoi* with 6.3–8.4 cm in diameter. The size comparison between our observation on the *ex-situ* cultivated living specimens and the literature found a considerably smaller fruit size in *D. celebica* only. However, the fruit shape comparison between our observation and the literature found some rounded and globose-shaped variations in *D. blancoi* and some ovoid-shaped variations in *D. blancoi*. Thus, the fruit variations of more flattened ones in *D. celebica* and the more elongated ones in *D. blancoi* could be considered as some new notes in the morphological variations of both species.

The population of *D. celebica* spreads across various locations in Sulawesi and creates some ecological varieties or provenance (Restu *et al.* 2017). Variability in tree stands that make up its population demonstrates that this species has a large morphological variation within species that is important for taxonomic identification and evolutionary studies. However, these variations remain understudied. On the other hand, *D. blancoi* has been widely cultivated for its edible fruits and fine-quality timber (Lemmens *et al.* 1995; Lim 2012). *D. blancoi* also varies naturally in leaf shape and fruit taste (Bakhuizen van den Brink 1938), but it is often selectively cultivated to produce large and sweet fruits (Hung *et al.* 2015).

4.2. Polymorphism and Heterozygosity

Information on polymorphism and the expected heterozygosity provided an estimation of the genetic diversity level in a population. High estimated values of both polymorphism and heterozygosity suggest high genetic diversity in the studied plant population. Through this study, plant polymorphisms in female/bisexual *D. celebica* and *D. blancoi* were both estimated to be considerably high > (86%) but substantially lower (< 55%) in male *D. celebica* (Table 2). Threatened and endemic plant populations might have low levels of genetic diversity. Such an example was reported in the case of agarwood-producing-tree species of (*Gyrinops versteegii* (Gilg) Domke) on Flores Island, whose polymorphism and heterozygosity value were at 55% and 0.218 respectively (Irsyad *et al.* 2020), almost similar to the level of genetic diversity of the male *D. celebica* analyzed in this study. Polymorphism and heterozygosity are highly influenced by the number of plants sampled within a population. A sufficient sample size is required to truly represent the genetic diversity in the studied population. This study analyzed 11 *D. celebica* out of 29 plants available at Purwodadi Botanical Garden. These samples are sufficient to obtain an accurate genetic diversity level of the *D. celebica* population at Purwodadi Botanical Garden.

Polymorphism in plants can be driven by several factors, including combined taxon-spatial-temporal preferences of pollinators in certain flower morphologies (Ortiz *et al.* 2015; Kellenberger *et al.* 2019; Hassa *et al.* 2020; Coetzee *et al.* 2021) and varying plant's morphological responses to environmental variables (Franks *et al.* 2013). Polymorphism in plants may also originate from correlated results of some other plant characteristics, such as flower forms, colorations, and chemistry, as well as flowering phenology and intensity (Narbona *et al.* 2018; Dormont *et al.* 2019; Kellenberger *et al.* 2019; Coetzee *et al.* 2021).

There has been no universal standard for level classification in heterozygosity values. However, some earlier studies classified heterozygosity values of approximately 0.20 as high for some plant families, especially for endemic plant species (Hamrick and Godt 1996; Finkeldey and Hattemer 2007; Allendorf 2017). Thus, this study's calculated heterozygosity of *D. celebica* and *D. blancoi* can be considered high for the female/bisexual population but moderate for the smaller population of male *D. celebica*. This result complied with previous studies that reported that gene diversity in a population was likely to be influenced by population size (Ellegren and Galtier 2016).

It has been generally expected that the genetic diversity of threatened or endemic plant populations is unlikely to be of high value. It is due to their substantially lower probability of random mating than the common or widely spread species whose population sizes are much bigger. However, several studies have reported varying estimates of expected heterozygosity (He) in threatened and endemic plant populations ranging from moderate to high. For instance, moderate He of 0.18–0.19 in an endemic orchid *Vanda foetida* from South Sumatra (Rindyastuti *et al.* 2015) and moderate He of 0.20 in an endangered rockrose Tuberaria major from Algarve, Portugal (Trindade *et al.* 2012) were reported. Additionally, a high He of 0.203 in a threatened *Dipterocarpus gracilis* population from Central Java (Romadini *et al.* 2021), while a low He of 0.117–0.141 in an endangered wattle *Acacia whibleyana* (Blyth *et al.* 2020) population from South Australia were also reported. Thus, the estimated genetic diversity level in *D. celebica* and *D. blancoi* populations cultivated *ex-situ* in Purwodadi Botanic Garden fell under the expected range of genetic diversity for threatened and endemic plant populations.

4.3. Implications for Plant Conservation in Botanic Gardens

Purwodadi Botanic Garden is an *ex-situ* plant conservation institution established to conserve Indonesian tropical lowland plant species. Maintaining enough individuals through assisted plant propagation is essential to the success of plant reintroduction programs (Volis 2016; Silcock *et al.* 2019). Another key success of plant conservation that needs to be considered is preserving high genetic diversity within the population to avoid deleterious inbreeding effects (Whitlock *et al.* 2016). Thus, information regarding the conserved species' phenotypic and genetic variability levels is vital for plant conservation (Coates *et al.* 2018). This research acquired information regarding phenotypic variability and estimated genetic diversity level in the living collections of an endemic *D. celebica* and a widely spread *D. blancoi* in the Purwodadi Botanic Garden. Our research highlighted the high phenotypic and genetic variability in female/bisexual specimens of both ebonies but moderate variability in the male *D. celebica* specimens. We suggest that the population size for both species needs to be maintained to preserve the already high phenotype and genetic variability in the population and to further improve them by adding more living collections from different subpopulations of Indonesia or by periodically performing human-assisted cross-pollination. However, the latter method requires careful consideration to prevent the occurrence of unnatural hybrids that might affect the preservation and conservation of the natural plant population.

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