

Morphological and genetic variation in populations of *Desmos chinensis* Lour. (Annonaceae)

ISNA AROFATUN NIKMAH^{1,2}, RUGAYAH RUGAYAH³, TATIK CHIKMAWATI^{4*}

¹Plant Biology Graduate Program, Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, IPB University Campus Dramaga, Bogor 16680, West Java, Indonesia

²Department of Biology, Faculty of Military Mathematics and Natural Sciences, Universitas Pertahanan Indonesia. Komplek Indonesia Peace and Security Center, Sentul, Bogor 16810, West Java, Indonesia.

³Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences. Jl. Raya Jakarta-Bogor Km. 46, Cibinong, Bogor 16911, West Java, Indonesia

⁴Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, IPB University Campus Dramaga, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8622833, *email: tatikch@apps.ipb.ac.id

Manuscript received: 6 January 2020. Revision accepted: 15 January 2021.

Abstract. Nikmah IA, Rugayah R, Chikmawati T. 2021. Morphological and genetic variation in populations of *Desmos chinensis* Lour. (Annonaceae). *Biodiversitas* 22: 811-822. *Desmos chinensis* is a woody climber or scandent shrub genus in Annonaceae that has moniliform fruits. *Desmos chinensis* Lour. can be found throughout Southeast Asia. This species is widely used as a traditional medicine in several countries in Asia, however, the information on its morphological and genetic variation has never been reported. The objective of this study was to reveal the genetic variation of *Desmos chinensis* on Java island (Indonesia) based on the morphological characters and Inter-Simple Sequence Repeats (ISSR) markers. Leaf samples were collected by exploring seven regions in Java island. Variation in morphological characteristics were described and discussed in detail. Genetic variation was assessed using the ISSR marker. The morphological characteristics of *D. chinensis* in Java varies in habit; leaf shape, leaf size, leaf color; flower position, flower reflexivity, pedicel bract position, number of petals, petal color, petal shape, petal apex, undulation of petal; and number of monocarp per fruit. A total of 53 samples collected from seven populations were screened by 25 ISSR primers, wherein 11 primers produced 47 clear and reproducible bands covering 85.1% of polymorphic bands. *Desmos chinensis* in Java were divided into five natural populations. Furthermore, the mean value of the Shannon index and genetic diversity index were 0.223 and 0.151, respectively. Moreover, the variations within and among populations examined using AMOVA showed the occurrence of 38% of total genetic variations among the different populations, therefore, the remaining 62% was due to variations within the population. Morphological and genetic variations are important preliminary information for determining the conservation strategy of *Desmos chinensis*.

Keywords: Analysis of molecular variance, AMOVA, genetic diversity, phenetic, population structure

INTRODUCTION

Desmos chinensis Lour. is a woody climber or spreading shrubs genus in Annonaceae characterized by chartaceous oblong or elliptic leaves, glaucous on the adaxial surface of leaves with acute to acuminate on the apex of leaves, prominent midrib and vein on the adaxial surface of leaves (Ng 2010; Turner 2012). The flowers of *D. chinensis* are single-pendulous flowered with six petals arranged in two whorls of petals (Turner 2012). Fruits of *D. chinensis* are moniliform containing globose or ellipsoidal seed (Ng 2010). *Desmos chinensis* is one of the species in Annonaceae which showed the greatest morphological variation and widest geographic distribution among species of *Desmos* (Sinclair 1955; Ng 2010; Turner 2012). Previous research of *D. chinensis* in Sumatra, Peninsular Malaysia, Thailand, and Borneo showed extensive variation in leaf size, pedicel length, petals, and fruit (Ng 2010).

Desmos chinensis is a medicinally, economically, and ecologically important plant species. In China, the bast fibers of *Desmos chinensis* are used for cordage and the leaves are used in brewing liquor in Hainan (Bingtao et al. 1994) while the flower contains an essential oil for

perfumery and the fruits are used to flavor food (Uphof 1959). *Desmos chinensis* is widely used as a traditional medicine especially in Indo-China, China, Thailand, and Peninsular Malaysia, to treat dysentery, vertigo, fever, and parturition (Lemmens 2003). Investigation to the biochemical properties of *D. chinensis* showed that the extracts of *D. chinensis* leaves have antimicrobial activity against human pathogens, including bacteria, yeast, and dermatophytic fungi (Kummee et al. 2008), and have the ability to inhibit the activity of tyrosine kinase which is responsible for cell growth and division in tumor cells (Kakeya et al. 1993). Ecologically, *D. chinensis* is a hostplant of butterflies such as *Graphium doson*, *Graphium agamemnon*, *Drupadia ravindra* (Easton and Pun 1997; Sulistyani 2013), beetle *Amystrops* (Pang and Saunders 2015), and oriental fruit fly of *Bactrocera dorsalis*.

Desmos chinensis is widely distributed in Asia and Australia. In Asia, *D. chinensis* can be found in India, Nepal, Bhutan, Bangladesh, China, Myanmar, Vietnam, Laos, Cambodia, Thailand, Malaysia, Singapore, Philippines, and Indonesia (Ng 2010; Turner 2015; Turner 2018). Indonesia is the country with the richest biodiversity in Southeast Asia, but unfortunately, the natural

environment in Indonesia faces a critical threat due to high population density and rapid rate of industrialization (Cleary and DeVantier 2001). Java Island has the most densely populated in Indonesia. As a consequence, fragmentation of natural forest in Java has extensively occurred. The last report, natural lowland forest in Java is only about 2.3% (Lambert and Collar 2002). Meanwhile, forests in the lowland of Java became the main habitats of various species of flora, including *Desmos chinensis*. The occurrence of *D. chinensis* in Java was first discovered in 1845 by Zollinger (on the label of *Zollinger 17728* in BO). However, the multipurpose utility of *D. chinensis* has not widely known by the people in Java and this can lead to the extinction of *D. chinensis*. Therefore, genetic conservation of *D. chinensis* is needed to reduce the risk of *D. chinensis* population extinction.

Genetic conservation of *D. chinensis* can be initiated by assessing the genetic variability and variation in morphological characters in the population of *D. chinensis*. These efforts are important for determining the best conservation strategies, maintenance of plant genetic resources, and breeding programs (Monfared et al. 2018). Genetic variation in population can be assessed using molecular markers (Afzal et al. 2018; Vinson et al. 2018). A molecular marker that has been widely used to investigate the genetic variation among populations is Inter Simple Sequence Repeat (ISSR) (Zietkiewicz et al. 1994).

Genetic variation at intraspecific and interspecific levels can be investigated using Inter Simple Sequence Repeat PCR-based marker (Wolfe and Liston 1998). The ISSR marker has several advantages. It does not require initial genome sequence information for primer design, low in costs, does not require complicated laboratory techniques, produce high polymorphism and repeatable, is able to differentiate between individuals within one species, and distinguish individuals with high morphological similarities (Zietkiewicz et al. 1994; Dagani et al. 2003; Aga et al. 2005; Okpul et al. 2005; Salhi-Hannachi et al. 2005; Tesfaye et al. 2014).

Previous studied on the *D. chinensis* population in China have shown that this species has a high level of genetic variability within population (Pang and Saunders 2015). However, there is no information on its morphological variation and genetic relationship within the species. Therefore, our study aimed to describe the morphological and genetic variation in the *D. chinensis* population. Moreover, this effort was carried out for the purpose of conservation on *D. chinensis* in the future.

MATERIALS AND METHODS

Plant materials

Plant materials consisted of 53 accessions that were collected from seven areas in Java Island: Trenggalek-East Java, Purwodadi Botanic Gardens-East Java, Batang, and Pekalongan-Central Java, Bogor Botanic Gardens-West Java (Figure 1). All accessions then were grouped into seven populations based on location origin. Accession from the same location was treated as the same population. Voucher specimens were deposited in the Laboratory of Ecology and Plant Resources, IPB University (Table 1).

Morphological measurements

The morphological characterization of vegetative and generative characters was observed based on Ng (2010). The observed characters were habit, plant height, leaf lamina shape, leaf lamina apex, leaf lamina base, leaf lamina color abaxially and adaxially, flower position, pedicel bract shape, sepal length, sepal width, sepal shape, outer petal length, outer petal width, outer petal shape, undulation of outer petal, outer petal apex, outer petal base, outer petal color, inner petal length, inner petal width, inner petal shape, inner petal undulation, inner petal apex, inner petal base, inner petal color, number of monocarp per fruit, monocarp length, monocarp width, and monocarp shape.

DNA extraction and ISSR amplification

Fresh leaves were extracted using Fast DNA TM SPIN Kit (MP Biomedicals, China). A total of 11 ISSR primers were used to amplify the DNA of seven *D. chinensis* populations (Table 2). The ISSR-PCR reactions were performed in 20 µL volume of reaction mixture containing 6.5 µL nuclease-free water (Thermo Fisher Scientific, USA), 12.5 µL 2X DreamTaq Green PCR Master Mix (2X), 1 µL bovine serum albumin, 1 µL formamide, 1 µL primer (10 µM), and 3 µL DNA. The reaction begins with an initial denaturation at 94 °C for 2 minutes, followed by 40 cycles of denaturation at 95 °C for 1 minute, primer annealing at 45-51 °C for 1 minute, extension at 72 °C for 1 minute, then final extension stage at 72 °C for 10 minutes. The PCR products were electrophoresed on 1.8% agarose gel in 1X TAE buffer stained with 5 µL peqGREEN 20 000X DNA/RNA binding dye (VWR, USA) at a voltage of 100 V for 60 minutes. The banding pattern was observed under UV light and photographed using a UV-transilluminator (WiseDoc, Germany).

Table 1. Accession code, location of origin, and population name

Accession code	Location	Population name	Population code	Province
NGE1-5	Ngembel, Watulimo, Trenggalek District	Ngembel	NGE	East Java
REJ1-5	Rejowinangun, Trenggalek, Trenggalek District	Rejowinangun	REJ	East Java
DON1-10	Dong Lurah, Trenggalek, Trenggalek District	Dong Lurah	DON	East Java
KRP1-4	Purwodadi Botanic Gardens	Purwodadi Botanic Gardens	KRP	East Java
BAT1-11	Adinuso, Subah, Batang District	Batang	BAT	Central Java
PEK1-4	Mesoyi, Talun, Pekalongan District	Pekalongan	PEK	Central Java
KRB1-14	Bogor Botanic Gardens	Bogor Botanic Gardens	KRB	West Java

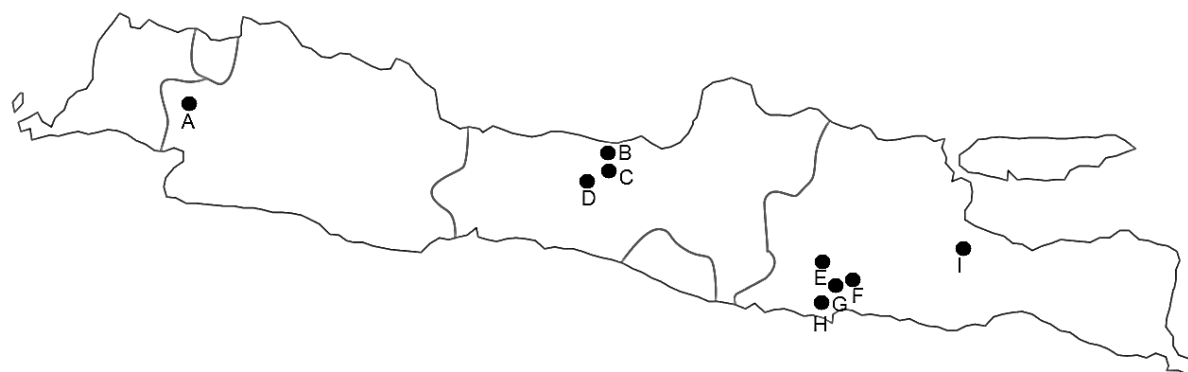


Figure 1. *Desmos chinensis* sampling location on the Island of Java, Indonesia. A. Bogor Botanic Gardens, B and C. Adinuso, Subah, Batang District, D. Mesoyi, Talun, Pekalongan District; E. Rejowinangun, Trenggalek District; F. Cemetery Area, Trenggalek District, G. Kedunglurah, Pogalan, Trenggalek District; H. Ngembel, Watulimo, Trenggalek District; I. Purwodadi Botanic Gardens

Table 2. ISSR primer, sequences, and their amplification profiles

Name	Sequence	Fragment size (bp)	Polymorphic bands	Total bands
ISSR G1	(GT) ₇ ACAG	300-1400	5	6
ISSR G2	(GA) ₈ A	250-1000	3	4
ISSR G3	(CT) ₇ C	500-1250	4	5
ISSR G4	(TG) ₇ CAC	250-1000	5	5
ISSR G5	(GA) ₇ CTC	750-1000	2	2
ISSR G8	(GT) ₈ T	250-1000	1	4
ISSR H3	(CT) ₈ T	500-1000	3	3
ISSR P1	(AGG) ₅	500-1200	3	4
ISSR P5	(GGGGT) ₃	250-750	4	4
ISSR P9	(GA) ₉ C	100-1500	8	8
ISSR P10	(GT) ₉ T	400-780	2	2
Total			40	47

Data analysis

Morphological characteristics were analyzed descriptively. A total of 53 samples were analyzed. Among 30 morphological characters, only 13 characters showed variation between *D. chinensis* populations in Java island. Sixteen features that do not indicate any variance were removed from the analysis. The phenetic analysis was performed using Sequential Agglomerative Hierarchical and Nested Clustering (SAHN) with Unweighted Pair Group Method with Arithmetic Average (UPGMA) method through the Numerical Taxonomy and Multivariate Analysis System for PC (NTSys-PC) version 2.1.1a (Exeter Software, New York).

The ISSR fragments were scored binary as present or absent. The size of ISSR fragments was estimated using DNA ladder 1 Kb. These binary scoring were then included in matrix data for generating tree topology based on the Tamura-Nei genetic distance coefficient. A dendrogram was constructed using SAHN with UPGMA method through the NTSys-PC version 2.1.1a (Exeter Software, New York).

The average logarithm of data similarity (likelihood) [LnP (D)] for estimating the ideal number of population was estimated using the STRUCTURE HARVESTER. Population structures were analyzed using STRUCTURE version 2.3.4 software. The molecular data were analyzed using R software. The number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), diversity (h), unbiased diversity (uH), and percentage of polymorphic loci (%P) were assessed for each population.

Variation among individuals within populations and variation among populations were analyzed using Analysis of molecular variance (AMOVA).

RESULTS AND DISCUSSION

Distribution and habitat type of *Desmos chinensis* in Java

Desmos chinensis was widely distributed in Java, i.e. Banten, DKI Jakarta, West Java, Central Java, and East Java, starting from the Westernmost region, Ujung Kulon to the Easternmost region, Banyuwangi (Figure 2). *Desmos chinensis* was not found in the province of DI Yogyakarta, Bandung (West Java), and Surabaya (East Java).

Desmos chinensis in Java inhabited many different types of habitats, including shaded area such as primary forest, secondary forest, mangrove forest, heterogeneous forest, limestone, and opened area such as along road, cemetery area (Figure 3), and garden, but they most frequently were found in the forest.

Desmos chinensis were distributed in the lowlands of Java Island with altitudes ranging from 3–600 masl (Figure 4). In all provinces in Java, *D. chinensis* could be found in nearly sea level elevation. In the province of West Java, Central Java, and East Java this species can be found in about 100 m asl.

Desmos chinensis was mostly collected in East Java and Central Java, less collected in DKI Jakarta and Banten. In East Java, *D. chinensis* was discovered in 1845 by Zollinger, in Lumajang. This species was mostly found in 1900–1950 in almost all provinces in Java, except Banten (Figure 5). In Banten province, *D. chinensis* was collected in 1963 and 1992. In several regions in East Java and Central Java, *D. chinensis* could be found from 1850–2019. The area in Central Java where *D. chinensis* had not been found since the 1800s was Baturraden Botanic Gardens, Purwokerto, which has about 700 m asl.

Morphological variations between accessions of *Desmos chinensis*

A total of 53 accessions of *D. chinensis* from the regions of East Java, Central Java, and West Java showed variations in the habit type, plant height, leaf shape, leaf

size, young leaf color, flower position, flower reflexivity, pedicel bract position, pedicel bract shape, outer and inner petal number, outer and inner petal color, outer and inner petal shape, outer and inner petal undulation, and monocarp per fruits number.

Habit

In Java, *D. chinensis* has two habit types, shrubs with up to 3 m tall, and lianas or climbing habit with up to 10 m long.

Leaf

Leaves of *D. chinensis* in Java vary in their shape, size, and color of adaxial surface. The leaf shape was elliptic and oblong. Leaf size of *D. chinensis* ranged from ca. 5.4–23 cm long. Morphological observations of the leaves of *D. chinensis* in Java also showed that the leaves of *D. chinensis* found in opened habitats were mostly smaller with light green color. The color of the young leaves of *D. chinensis* varies from slightly reddish, light green, to dark green. The reddish or light green young leaves were generally found in populations that grow in the opened area with high intensity of sunlight, such as on roadsides, plantations, and cemetery area. Meanwhile, dark green leaves were found in *D. chinensis* populations, which generally grow in shaded areas such as in secondary forests (Figure 6).

Flower

Desmos chinensis flowers vary in the position of the flower, reflexed or not reflexed flower, the position of pedicel bract; number, color, shape, apex, and undulation of petals. The flower position of *D. chinensis* varies from supra-axillary (above the axillary) to sub-leaf-opposed (slightly opposite to the leaf), and extra-axillary (close to the petiole). The most common variation of flower position was supra-axillary, but the position of flower varies among individuals of *D. chinensis* in a population. The population of *D. chinensis* in the Purwodadi Botanic Gardens has reflexed flowers (Figure 7), while the population of Ngembel and Bogor Botanic Gardens have erect flowers.

The position of pedicel bract varies, on the lower sub-median of pedicel, and upper sub-median of pedicel. The shape of pedicel bract also varies from an ovate to broadly ovate. The number of petals in *D. chinensis* flower was three outer petals and three inner petals. However, the individual *D. chinensis* from the Ngembel population have seven petals. The color of petals varied from green, yellow, to reddish yellow (Figure 7). However, the green color of *D. chinensis* flowers could be caused by development stage. Young flowers were generally green, while mature flowers were yellow or reddish. The shape of petals varies from obovate, lanceolate, and elliptic. The apex of petals varies from acuminate and obtuse. The margin undulation of petals were sinuate and undulate (Figure 7).

Fruit

The fruits of *D. chinensis* varied in the number of monocarp per fruit. The number of fruit monocarp of *D. chinensis* ranged from 4 to 24 monocarp per fruit.

Cluster analysis of morphological characters

We examined 25 morphological characters of 53 accessions of *D. chinensis*. The results showed that all accessions of *D. chinensis* were separated into 2 main groups at the similarity coefficient of 0.78. Group I consisted of the Ngembel population, some individuals of Donglurah population, Batang population, and Pekalongan population. Group II consisted of the Rejowinangun population, Bogor Botanic Gardens population, some of Donglurah population, and Purowdadi Botanic Gardens population (Figure 8). These two groups were separated mainly by habit character, leaf lamina apex, leaf base, leaf lamina color adaxially. The habit of group I was shrubs, while the habit of group II was liana. The adaxial surface color of the leaf lamina of group I was mostly light green, while group II was mostly dark green.

Group I was divided into two subgroups: Group A and Group B. Group A consist of the Ngembel population, some of Batang population, Pekalongan population, and some of Donglurah population. Group B consist of some of Donglurah and some of Batang population (Figure 8). These groups were separated by leaf lamina shape. Leaf lamina shape of Group A was mostly oblong, while group B was mostly elliptic. However, the leaf lamina shape of *D. chinensis* was highly variable among individuals and the population. An individual of *D. chinensis* exhibited two leaf lamina shapes.

Group II was divided into two subgroups: Group C and Group D. Group C consist of the Rejowinangun population, Bogor Botanic Gardens population, some accessions of Donglurah population, while Group D consist of the Purwodadi Botanic Gardens population. These two groups were separated by outer petal length, outer petal undulation, outer petal reflexed or not reflexed, inner petal undulation, inner petal apex, and inner petal reflexed or not reflexed. These characters were the important character which distinct constantly among these groups.

Genetic variations between *Desmos chinensis* accessions based on ISSR markers

ISSR amplification of 53 accessions of *D. chinensis* using 11 selected primers produced 47 markers, of which, 40 (85.1%) were polymorphic across all samples. The number of amplified fragments varied from 2 (by G5 and P10 primers) to 8 (P9 primer). Sizes of the amplified fragments ranged between 100-1500 base pairs (bp) (Table 2).

Tamura-Nei coefficient was used to access the relationship between *D. chinensis* population in Java. The 53 accessions of *D. chinensis* were clustered based on their geographical regions, although there were some individuals from the same location that have not occurred in the same group. Clustering based on molecular data showed differences with the clustering analysis using morphological data. Genetic relationship analysis showed that most of the individuals from the population belongs to East Java province (except Donglurah and Purwodadi Botanic Gardens) genetically related with the population belongs to Central Java province. A dendrogram generated using the ISSR data clustered the 53 accessions of *D. chinensis* into two major clusters. Group I contain of all

individuals from the Ngembel population, Rejowinangun population, Batang population, Pekalongan population, and some individuals from Donglurah population and Bogor Botanical Garden population. Group II contains of most individuals from the Donglurah population, all individuals from the Purwodadi Botanic Gardens population, and some individuals from the Bogor Botanic Gardens population. One population that was in the same cluster as another population indicates that both have a similar genetic structure. Thus, the population of East Java showed a similar genetic structure to the population of Central Java.

The two main groups of *D. chinensis* were further divided into seven groups. The Ngembel and Rejowinangun populations were in group 1. Some individuals of Batang forms occurred in the same group with two individuals from Bogor Botanic Gardens into group 2. Other individuals from the Bogor Botanic Gardens population clustered into group 3. Other individuals from Batang and Pekalongan populations were in group 4. Some of the individuals of the Donglurah population clustered into group 5 and the other Donglurah individuals were clustered into group 6. The remaining cluster (group 7) contains some individuals from Bogor Botanic Gardens (Figure 9).

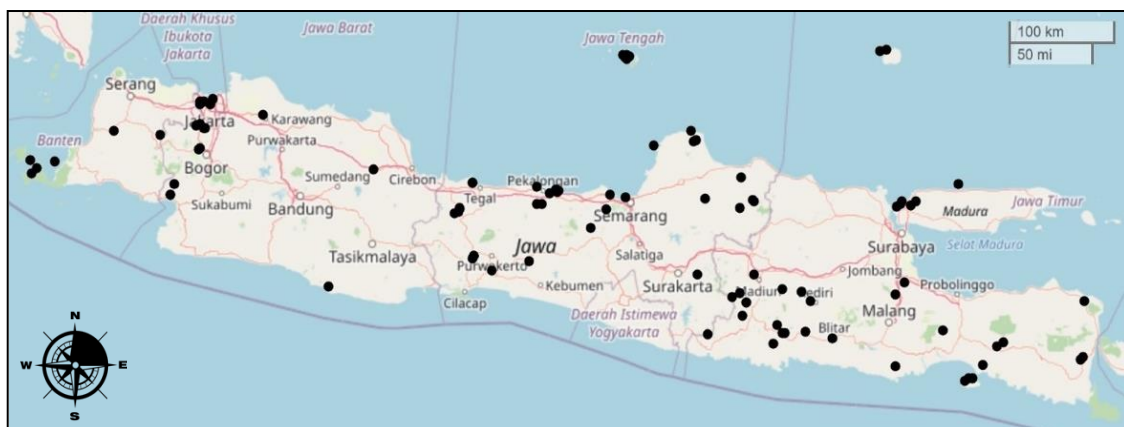


Figure 2. Geographical distribution of *D. chinensis* based on herbarium specimens



Figure 3. Habitat of *Desmos chinensis* in Java, Indonesia. A. *D. chinensis* grew in shaded area in East Java, B. *D. chinensis* grew in *Albizia chinensis* plantation in Central Java, C. *D. chinensis* found in cemetery area associated with *Plumeria* in East Java, D. *D. chinensis* found along road in Central Java

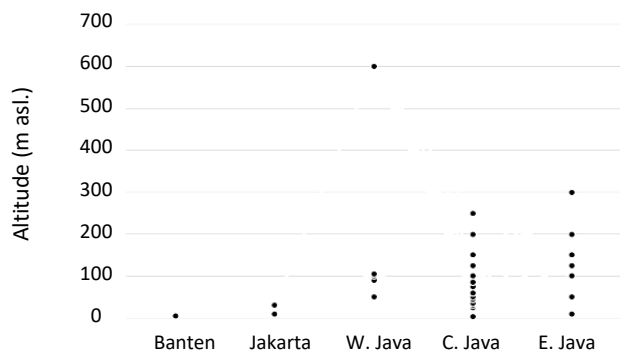


Figure 4. Elevation range of *D. chinensis* in Java, Indonesia

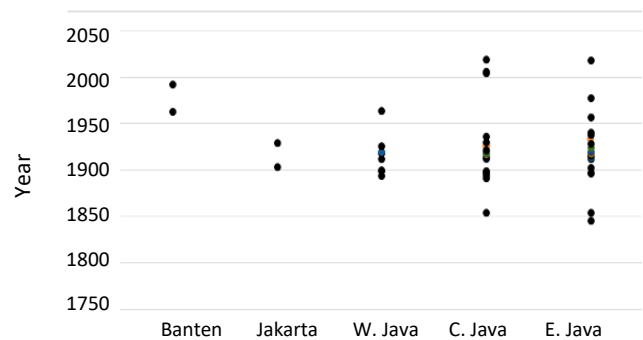


Figure 5. Years found *D. chinensis* in Java, Indonesia



Figure 6. Leaf of *Desmos chinensis*, light green leaves with reddish color on young leaves of *D. chinensis* found in opened area with high intensity of sunlight (A), dark green leaves of *D. chinensis* grow in shaded area



Figure 7. *Desmos chinensis* flower, reflexed green petals and undulate margin (A), yellow obovate, with obtuse apex and not reflexed outer petals (B), reddish yellow petals with undulate margin (C), yellow lanceolate with sinuate undulation and acuminate apex of petals (D). Scale bar = 5 mm

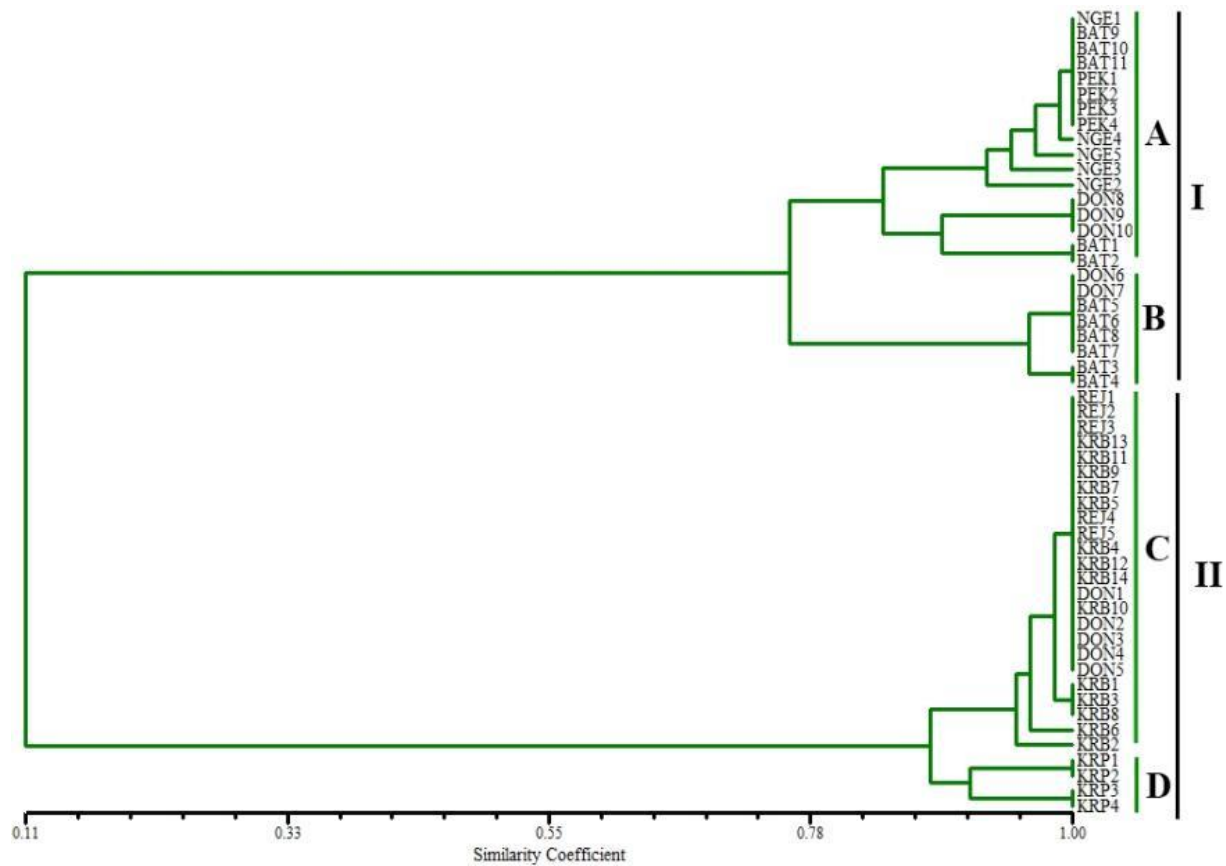


Figure 8. Dendrogram of 53 *Desmos chinensis* based on morphological data

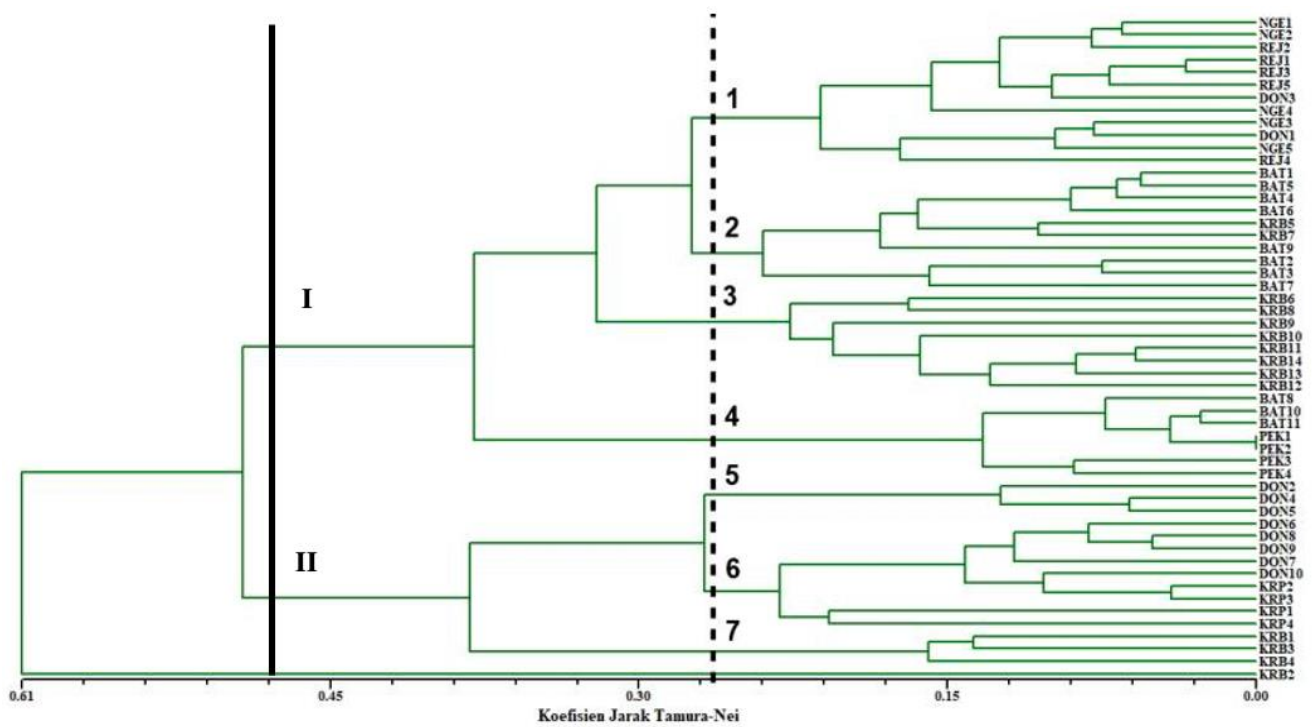


Figure 9. Dendrogram of 53 *D. chinensis* individuals based on ISSR data analyzed by the UPGMA method

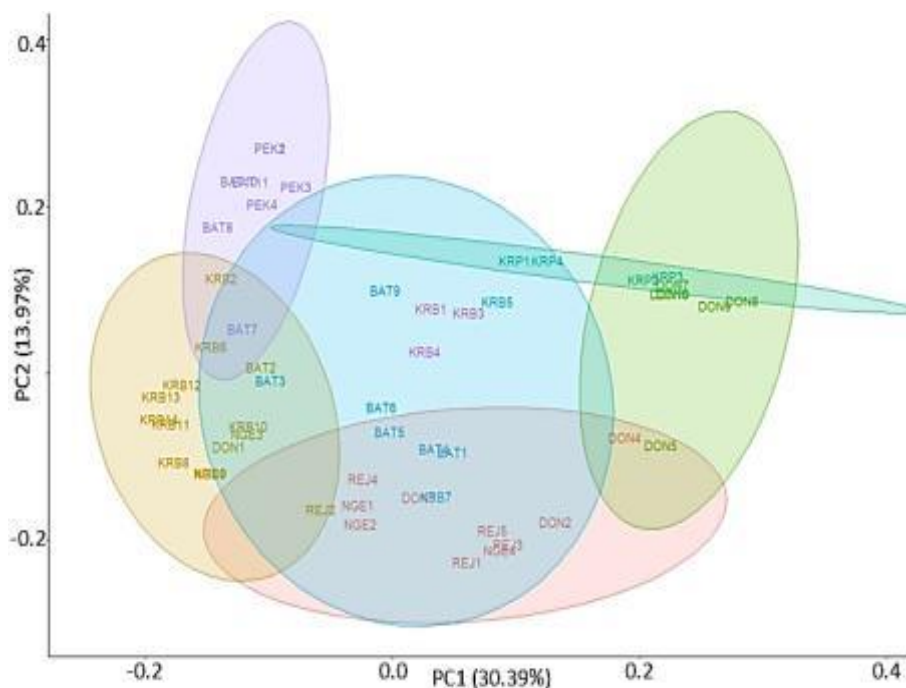


Figure 10. Plot matrix in Principal Coordinate Analysis (PCoA) based on ISSR data

Table 3. Genetic parameters and polymorphic diversity of *Desmos chinensis* loci

Population	N	Na	Ne	I	h	uh	%P
Ngembel	5.000	1.233	1.043	0.200	0.136	0.170	34.78%
Rejowinangun	5.000	1.192	1.000	0.171	0.115	0.143	30.43%
Dong Lurah	9.978	1.543	1.408	0.352	0.236	0.263	65.22%
Purwodadi	4.000	1.183	0.848	0.155	0.106	0.141	26.09%
Batang	11.000	1.317	1.217	0.260	0.178	0.195	45.65%
Pekalongan	4.000	1.083	0.543	0.067	0.046	0.062	10.87%
Bogor	14.000	1.457	1.422	0.355	0.241	0.260	63.04%
Mean	7.568	1.286	1.068	0.223	0.151	0.176	39.44%

N: Samples Size; Na: Number of Alleles; Ne: Number of Effective Alleles; I: Information Index; h: Diversity; uh: Unbiased Diversity; %P :Percentage of Polymorphic Loci

Table 4. Analysis of molecular variance of seven *Desmos chinensis* populations using ISSR markers

Variation source	df	SS	MS	%	PhiPT	Prob.
Among population	6	157.201	26.200	38	0.382	0.001
Within population	46	219.702	4.776	62	-	-
Total	52	376.903	-	-	-	-

Genetic relationships within and between populations were assessed using Principal Coordinates Analysis (PCoA). PCoA was inconsistent with those clustered by UPGMA and separated *D. chinensis* into six groups. All individuals from the Pekalongan population were grouped and have similarities with some individuals from the Batang population. Likewise, the population of Purwodadi

Botanic Gardens was one group. Most of the Bogor Botanic Gardens population was grouped to one group, although some individuals were similar to the Batang population. The population from East Java has similarities with one another, consisting of the Rejowinangun, Ngembel, and Dong Lurah populations (Figure 10).

The delta K analysis of mean posterior probability [LnP (D)] showed that the highest likelihood values [LnP (D)] were determined for K = 5 (-650) (Figure 11a), meaning that *D. chinensis* from Java constitute five genetic clusters or there were five genetically different populations of *D. chinensis* in Java.

The clustering by the UPGMA method to all accessions showed no resemblance to the result by STRUCTURE. The five main groups were depicted in five different colors in the form of a bar chart based on the results of the analysis using the STRUCTURE program. The colors that dominate this chart were dark blue, red, purple, green, and yellow. At K = 7 most of the individuals were not clustered into a

specific group except for the Bogor Botanic Gardens samples (clustered into the same group: the red group), the Pekalongan samples (clustered into the same group: the blue group), Purwodadi Botanic Gardens samples (clustered into the same group: the orange group), and the Donglurah samples (clustered into the same group: the orange group) (Figure 11.B).

The mean values of the number of different alleles (N_a) and the number of effective alleles were 1.286 and 1.068, respectively. These seven populations have mean values for I , h , and u_h of 0.233, 0.151, and 0.176, respectively. Of the seven populations, the Donglurah population ($I = 0.352$, $h = 0.236$, $u_h = 0.263$) and the Bogor population ($I = 0.355$, $h = 0.241$, $u_h = 0.260$) were the most diverse populations, while the Pekalongan population was less diverse ($I = 0.067$, $h = 0.046$, $u_h = 0.062$). The percentage of polymorphic loci per population ranged from 10.87% (Pekalongan) to 65.22% (Donglurah) with a mean of 39.44% (Table 3).

The results of the molecular variance analysis of seven populations of *D. chinensis* in Java showed that most of the species diversity was within the population (62%), and the other variations were caused by differences between populations ($\Phi_{PT} = 0.382$, $p = 0.001$) (Table 4). The greater the Φ_{PT} value, the greater the difference between populations.

Discussion

Desmos chinensis is the only species of genus *Desmos* that is widely distributed in Java. Java is one of the mainlands in Indonesia, with the largest population (Bemmelen 1949). Therefore, research on plant diversity will be very useful for the conservation of germplasm.

Research on Annonaceae in Java is still limited. Most of the Annonaceae were conserved in the ex-situ conservation site, such as in the Bogor Botanic Gardens (Handayani 2018) and Purwodadi Botanic Gardens (Lestari et al. 2017). Inventories of Annonaceae in Java have not been reported again after the work of Backer and van den Brink (1963).

In this study, we explored *Desmos chinensis* at several locations in Java Island based on the specimen of Backer with/without Bakhuisen van den Brink deposited in BO, then mapped the distribution of *D. chinensis* based on the Herbarium specimen records (Figure 2). *Desmos chinensis* was found in almost all cities/districts in Java, except Yogyakarta, Bandung (West Java), and Surabaya (East Java) (Figure 4; Figure 5). The absence of *D. chinensis* in these areas could be related to the microclimate and elevation, in which Bandung is an area dominated by an altitude of up to about 800 m or 2624 ft asl, higher than some areas where *D. chinensis* was found in Central and East Java. Research on Central African Annonaceae recorded that Annonaceae can grow in low and mid-elevations (lowland: sea level to 700 m and submontane: 800 to 1800 m) (Bele et al. 2011). *Desmos chinensis* in China grows at an elevation of 100–1500 masl (Bingtao et al. 1994). The absence of *D. chinensis* at an elevation of more than 600 masl indicates that the presence of *D. chinensis* could be influenced by other abiotic factors, such

as soil pH and rock cover (Yang et al. 2018). Studies of liana in Taiwan reported that these factors were related to different topographic sites (Yang et al. 2018).

Desmos chinensis found in various habitats, grouped as shaded areas, and opened areas, based on the intensity of sunlight on the area (Figure 3). Shaded areas comprised such as secondary forest, and opened areas comprised such as along the road. Liana or woody climber species is an important component of the forest, but their occurrence depends on abiotic factors, such as total rainfall, seasonality of rainfall, soil fertility, and disturbance (Schnitzer and Bongers 2002). *Desmos chinensis* was abundant in forests presumably because as a woody climber species, this species requires other trees for its hosts. Liana species richness was higher in the primary forest than in secondary forests and distributed by an animal such as birds (Yuan et al. 2009). In China, *D. chinensis* lives in the Wastelands and thickets in valleys (Bingtao et al. 1994).

Desmos chinensis was first discovered on the island of Java by Zollinger, around 1845, in East Java. Over time, this species also continues to be found today. This means that for about 175 years, this species could still adapt to environmental changes in several areas in Java such as Central Java and East Java. In DKI Jakarta province, herbarium records showed that the last discovery of *D. chinensis* was in around the 1950s. This was thought to be due to the increasing human activity, including the expansion of residential areas and the increasing development related to the status of Jakarta as the capital city of Indonesia.

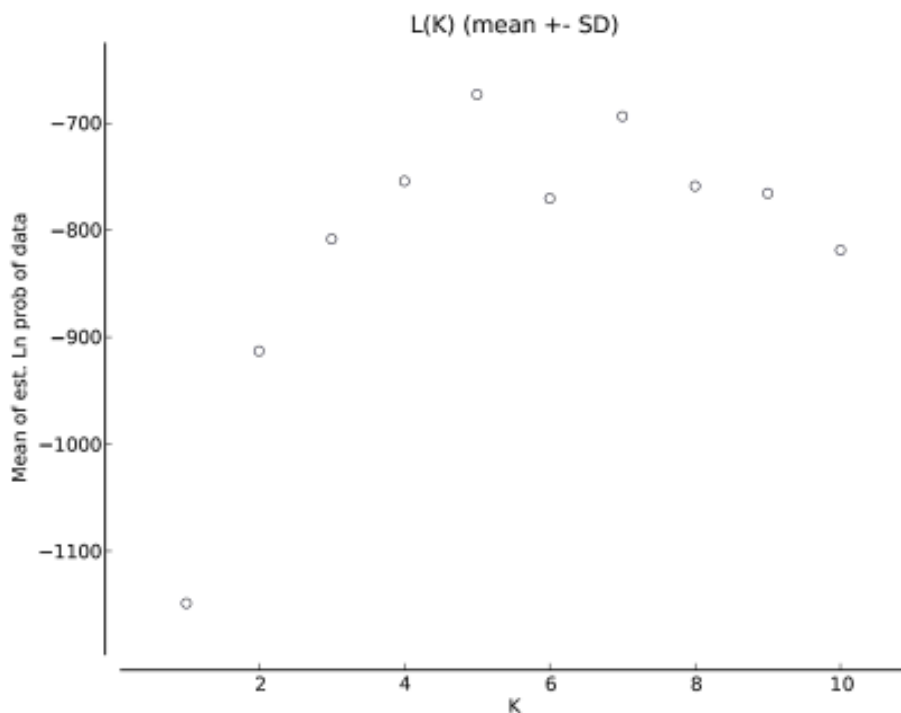
As a plant that has successfully adapted in Java, *D. chinensis* has developed morphological variations as part of the adaptation process to the environment. *Desmos chinensis* in Java can be found in two types of habit, including climber and shrub. According to Gianoli (2015), in order to grow vertically and improve light acquisition, climbing plants need external support. Therefore, if there were external support, then *D. chinensis* grows as a climber, but when there is no external support, it grows as a shrub. Yang et al. (2018) stated that the size of the climbing species can be affected by the size of the tree as the hosts of liana. Larger lianas were always found on larger host trees, while smaller lianas were found in smaller trees (Yang et al. 2018).

Leaf shape (1), leaf size (2), and leaf color (3) of *D. chinensis* also varied (Figure 6; Figure 7). (1) The study of leaf shape formation by Dkhar and Pareek (2014) reported that leaf shape formation is inherently genetic. In addition, the final adjustment of leaf shape is influenced by environmental factors. (2) A previous study on the correlation between abiotic factors and leaf reported that decreasing leaf size can be caused by increasing altitude, decreasing rainfall, and soil nutrient content (McDonald et al. 2003), and also smaller-sized leaves are used to adapt to hot or dry environments. However, leaf sizes of *D. chinensis* are highly varied, thus it cannot be used to differentiate among *D. chinensis* populations. (3) The leaf color is related to daily sunlight. The amount of light intensity will affect the production of sugars during

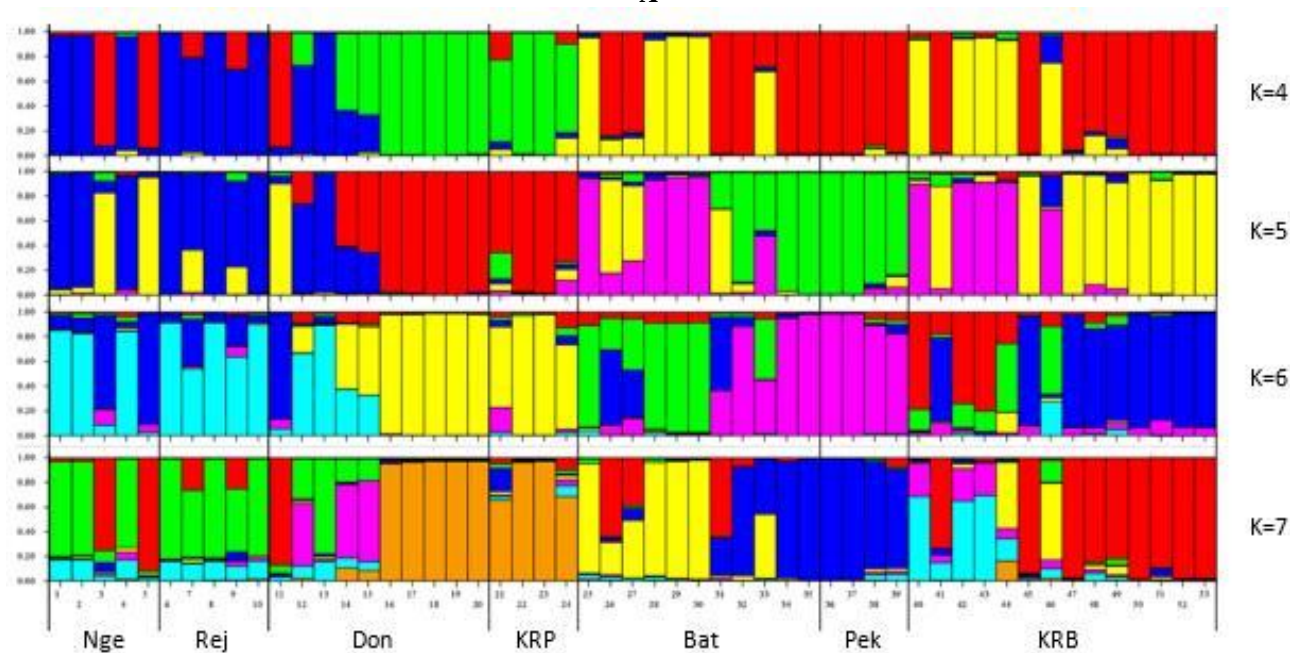
photosynthesis. If the sunlight intensity is low, the production of sugars begins to slow down, and chlorophyll will be reduced then leaves will be in green color. As a decreasing chlorophyll production, the other pigments are enhanced in the leaves. Meanwhile, the reddish color of leaves in *D. chinensis* that grow in the opened area may be explained by the discovery from Givnish (1988) that the red color of leaves is related to temperature and moisture

conditions before and during the period when the amount of chlorophyll in the leaves is decreasing.

The flowers of *D. chinensis* in Java is also vary in the position of the flower, reflexed or not reflexed flower, the position of pedicel bract; number, color, shape, apex, and undulation of petals. Reflexed flowers can be found in the population of Purwodadi Botanic Gardens. This variation is also found in the *D. chinensis* population in China (Pang and Saunders 2015).



A



B

Figure 11. Population structure of *Desmos chinensis* in Java Island, Indonesia. Plot of mean posterior probability (LnP(D)) values per clusters (K), based on 10 iterations per K (A) and structure analysis of *Desmos chinensis* in Java (B)

The number of petals in *D. chinensis* found ranged from six to seven. Seven petals rarely occur in *D. chinensis* (Backer and van den Brink 1963). Moyroud and Glover (2017) state that ETTIN/AUXIN RESPONSE FACTOR (ETT/ARF3) plays a role in increasing the number of petals in the *Arabidopsis* flower. Mutation in the ETTIN/AUXIN RESPONSE FACTOR (ETT/ARF3) gene that mediates auxin signaling led to the production of larger *Arabidopsis* flowers with extra sepals and petals but fewer stamens (Moyroud and Glover 2017).

The flowers of *D. chinensis* in Java are found in two different colors of the mature flower, i.e. yellow flowers and red flowers. Yellow flowers were found in the Bogor Botanical Garden population, while the red flowers were found in the Ngembel population. Pang and Saunders (2015) reported that in the pistillate phase, the petals of *D. chinensis* will change color to yellow and red at the base of the petals close to the reproductive organs. However, the yellow flowers of *D. chinensis* in Bogor Botanical Garden have never turned red. The slightly reddish color of the petals in adult flowers was also found in the *D. chinensis* population in China, with reflexed petals (Pang and Saunders 2015), on the other hand, the petals of the Ngembel population are reddish but not reflexed.

The shape of petals was also varied. According to Ng (2010), the shape of petals can be a distinguishing feature of *Desmos* species. Clustering *D. chinensis* using morphological character and molecular character were weakly correlated. Clustering using morphological data was not consistent with the molecular data. It can be seen that molecular divergences were not accompanied by morphological changes. The rate of evolution of molecular features was not in line with the rate of evolution of morphology. Molecular evidence indicates that, despite some morphological variation on the habit, plant height, leaf shape and size, young leaf color, flower position, flower reflexed, pedicel bract position, pedicel bract shape, outer and inner petal number, outer and inner petal color, outer and inner petal shape, outer and inner petal undulation, and monocarp per fruits number, suggesting that all of these morphological variations should be considered as a single species, *D. chinensis*.

Analysis of population structure was interpreted by the color of the bar from the result STRUCTURE program. The size of the color of the bar that was longer than the size of the other colors on each block indicates the dominant population. The similarity of dominant color variations in different populations indicates that these populations have close genetic characteristics. The ratio of several colors of the same length in each population is rarely seen in the STRUCTURE analysis results of *D. chinensis*, meaning that each population was dominated by several individual genotypes (Figure 11b). The mixing of colors in several individual genotypes indicates a flow of genes between populations caused by genetic mixing (Wicaksono 2017).

Analysis of genetic variation in *D. chinensis* shows that this species has high genetic variation based on the mean value of the Shannon index (I) and genetic diversity index (h), and percent of polymorphism (Tabel 3). Ayala and

Kiger (1984) reported that species with high genetic variation will be able to survive in a long time to environmental pressure.

The analysis of molecular variance indicated that variation within the population was higher than variation among the population of *D. chinensis*. Hamrick et al. (1992) stated that long-lived woody plants usually retain a greater percentage of their variation within the population. They reported that outcrossing plant species tend to have 10–20 % of the genetic variation among populations, whereas selfing species have, on average, 50 % of the variation among populations. *Desmos chinensis* showed the percentage of variation within the population is greater (62%) than variation among the population (38%), indicated that *D. chinensis* is an outcrosser (Table 4). This result is consistent with the results of research on the genetic diversity of the *D. chinensis* population in China (Pang and Saunders 2015). Studies on the biology of flowers in the population of *D. chinensis* in China state that *D. chinensis* is a species that tends to cross-pollinate despite having hermaphrodite flowers (Pang and Saunders 2015).

Clustering with morphological characteristics classifies *D. chinensis* into two main groups based on differences in vegetative characteristics, one of which is a habit, and one of the generative features is differences in flowers. However, despite being different, these two groups cannot be classified under a specific level. Molecular features support this data. Variation within a species of *D. chinensis* may an ongoing to be varieties, but it may take a long time.

Desmos chinensis in Java has high morphological and genetic variations. *Desmos chinensis* is distributed in almost all regions of Java island on the lowland area and various habitats, both opened and shaded habitat. Morphological variation clustering is not positively correlated with molecular clustering. It is estimated that there are five natural populations of *D. chinensis* that mostly consist of several genotypes in each population. The genetic variation of *D. chinensis* within a population has a greater value than the genetic variation among populations.

ACKNOWLEDGEMENTS

The author would like to thank Bogoriense Herbarium (BO), Sandakan Herbarium (SAN), Singapore Herbarium (SING) for allowing us to observe herbarium specimens, and Prof Mien A. Rifai for giving us valuable comments in preparing this manuscript. Thanks also to the PMDSU scholarship from Kemenristek-DIKTI for financial support.

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