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Similarity level of *Nepenthes* spp. based on the qualitative characters

Putri Lukmanasari[1*], Aziz Purwantoro[1], Rudi Hari Murti[1], and Zulkifli[2]

[1]Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora no. 1, Bulaksumur, Sleman, Yogyakarta 55281, Indonesia
[2]Faculty of Agriculture, Islamic University of Riau
Jln. Kaharuddin Nasution 113, Pekanbaru Riau 28284, Indonesia
*Corresponding author: putrilukmanasari@mail.ugm.ac.id

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ABSTRACT

Nepenthes, also known as pitcher plant, are unique and interesting flora developed as ornamental plants. This species has an attraction not only on the flowers but also on the diverse shapes and colors of the pitchers. Each Nepenthes species and hybrid has a variety of characters. The genetic relationship and diversity in Nepenthes species and hybrids can be determined based on the morphological characterization. The purpose of this study was to determine the similarity level among the Nepenthes accessions based on 31 qualitative characters. In this study, 30 Nepenthes species and 11 hybrids were used. The range of the similarity coefficient of Nepenthes species was 0.28–0.65, and divided into two major groups namely group A and B. Group A was the Nepenthes population which had petioles consisted of 17 accessions, while group B had folius consisting of 24 accessions. It indicated that when the similarity value is higher, the similarity level between each plant becomes higher. Principle Coordinates Analysis (PCoA) provided an overview of the relationship among the genotypes in coordinates. Species with a high similarity coefficient were in a close position, whereas species with a low similarity coefficient were in a remote position. Based on the PCoA, the species were grouped based on the similar characters, which was supported by the dendrograms.

INTRODUCTION

Nepenthes also known as pitcher plants are unique and interesting flora mostly developed as ornamental plants. These plants are attractive not only on the flowers but also on the various shapes and colors of the pitchers. There are approximately 82 types of Nepenthes in the World (Jebb and Cheek, 1997). According to Mansur (2006), there are 64 types of Nepenthes in Indonesia including in Sumatra and Borneo. There are approximately 29 types of Nepenthes in Sumatra as one of the centers of Nepenthes diversity after Borneo. Meanwhile in Borneo, there are approximately 32 types of Nepenthes. The use of Nepenthes as ornamental plant is very popular in overseas and more than 280 hybrids of Nepenthes have been produced through either artificial or natural crossbreeding.

Based on Law Number 5, year 1990 concerning Conservation of Natural Resources and Ecosystems (Cabinet Secretariat of the Republic Indonesia, 1990) and Government Regulation Number 7, year 1999 concerning the preservation of wild plants and animals (Cabinet Secretariat of the Republic Indonesia, 1999), Nepenthes is classified as a protected plant. The habitat destruction results in the inclusion of Nepenthes species in the Convention on International Trade in Endangered Species (CITES) data or in the endangered category of the Redlist-International Union for Conservation of Nature and Natural Resources

(IUCN) (International Union for Conservation of Nature and Natural Resource, 2020). The global protection status of Nepenthes in CITES is categorized in Appendix I for *N. rajah* and *N. khasiana*, and Appendix II for other Nepenthes species (Convention on International Trade in Endangered Species, 2020). The limited and damage of the forest area will have a direct impact on Nepenthes population and diversity decline. It can even lead to extinction (Wardani, et al. 2015).

Plant characterization is the process of finding specific characters of plants to determine each type among species. The plant characterization aims to produce plant descriptions. Each cultivar can have similarities or differences in characteristics. The morphological characteristics can be used to determine the close relationship between the nepenthes cultivars. The more similar the characters the species have, the closer their genetic relationship is. Conversely, the more different the characters the species have, the more distant their genetic relationship is (Mustofa et al., 2013).

Nowadays, the characterization of pitcher plants is largely based on morphology. Tjitrosoepomo (2018) stated that plant morphology is the study of the external shapes and organs of plants, making it possible to visually distinguish the plants. vHowever, morphological characters are usually confusing in their classification since there are many environmental factors that can be misinterpreted. Although the morphological markers are still widely used to control the success of a crossbreeding process, these markers are considered to be an unstable. In addition, morphological markers are very limited in number, and the observations are highly dependent on the time of the plants' life phase. Morphological markers are used to indicate the qualitative and quantitative characters. In general, qualitative characters are not influenced by the environment and more presented by visual information. On the other hand, quantitative character is basically strong,influenced by the environment. Environmental influences greatly affect the expression of genotypes. The advantage of quantitative character data is that they are more representative in describing genotype performances and are measurable because they have units. Quantitative characters in plants are generally controlled by many genes and are influenced by environmental factors (Maryono et al., 2019).

This study used a qualitative character observation since the qualitative characters of variability were narrow and insignificantly influenced by the environment, so that the similarity analysis by involving qualitative characters through phenotypic appearance was correlative through molecular analysis approaches. Qualitative characters as the manifestations of phenotypes differed from each other and were grouped in different categories. On the other hand, the reason for not using quantitative data in similarity level was because the use of quantitative data might cause misinterpretation of the grouping of plant population of different species. The error was caused by the existence of ecological and evolutionary factors affecting the development of a species. Ecological factors influenced quantitative characters more, so that these characters were overlapping (Westoby et al., 1995).

Morphological markers of Nepenthes had been widely used in genetic diversity studies, including *N. gymnamphora* and *N. mirabilis* (Iqbal and Susandarini, 2015), *N. ampullaria*, *N. gracilis*, *N. rafflesiana* and *N. mirabilis* (Selviana et al., 2018), *N. ampullaria*, *N. tobaica*, and *N. mirabilis* (Mukra et al., 2018), N. *gymnamphora* (Putra and Fitriani, 2018), *N. grancilis* Korth., *N. maxima*, *N. refflesiana* Jack., and *N. mirabilis* (Cahyono et al., 2019). The use of morphological markers to evaluate the genetic diversity of Nepenthes in Indonesia was still limited in some populations such as *N. gracilis* Korth, *N. rafflesiana* Jack, *N. mirabilis*, *N. khasiana*, and *N. ampullaria*. This information will be useful in adding information about Nepenthes in the future plant breeding program.

MATERIALS AND METHODS

This research was carried out at Yagiza Nursery, Insectivorous Plants Nursery, Tulungagung Nepenthes Community, and Venom Nursery in April–May 2019. The materials used in this research were 41 species of hybrid pitcher plants obtained from an exploration at four sampling sites (Table 1).

The observation of plant samples based on morphological characters was to describe the morphological character in the descriptors. Exploration activities began by observing Nepenthes accessions qualitatively. The observations of plant samples were based on qualitative characters covering the

Table 1. List of pitcher plant species and hybrid morphologically characterized

Codes	Pitcher plant types	Origins/Distributions	Nurseries
N29	*N. Jacqueline*	Sumatra	Tulungagung Nepenthes Community
N34	*N. longifolia*	Sumatra	Venom Nursery
N36	*N. tobaica*	Sumatra	Yagiza Nursery
N38	*N. adnata*	Sumatra	Yagiza Nursery
N40	*N. adrianii*	Sumatra Java	Tulungagung Nepenthes Community
N15	*N. eustachya*	Sumatra	Yagiza Nursery
N28	*N. beccariana*	Sumatra	Yagiza Nursery
N27	*N. sumatrana sibolga*	Sumatra	Yagiza Nursery
N16	*N. reinwardtiana*	Sumatra Borneo	Tulungagung Nepenthes Community
N4	*N. ampullaria*	Borneo Sumatra Thailand Malaysian Peninsula Singapore Maluku Islands New Guinea	Venom Nursery
N32	*N. rafflesiana*	Borneo Sumatra Malaysian Peninsula Singapore	Insectivorous Plants Nursery
N6	*N. gracilis*	Borneo Cambodia Malaysia Singapore Sulawesi Sumatra Thailand	Venom Nursery
N41	*N. kampotiana*	South of Cambodia East of Thailand West of Vietnam.	Insectivorous Plants Nursery
N7	*N. mirabilis*	Borneo Cambodia Malaysia Singapore Sulawesi Sumatra Thailand	Venom Nursery
N8	*N. globosa*	Thailand	Venom Nursery
N5	*N. bicalcarata*	Borneo	Venom Nursery
N17	*N. clipeata*	Borneo	Yagiza Nursery
N25	*N. northiana*	Borneo	Yagiza Nursery
N26	*N.veitchii*	Borneo	Yagiza Nursery
N45	*N. hirsute*	Borneo	Yagiza Nursery
N44	*N. albomarginata*	Borneo Malaysian Peninsula Sumatra	Venom Nursery
N42	*N. xhookeriana*	Borneo Malaysian Peninsula Singapore Sumatra	Yagiza Nursery
N30	*N. eymae*	Sulawesi	Yagiza Nursery
N48	*N. undulatifolia*	Sulawesi	Yagiza Nursery
N49	*N. danseri* or *N. spathulata*	Maluku Islands	Yagiza Nursery
N52	*N. maxima Maluku*	Sulawesi Maluku Papua	Yagiza Nursery
N43	*N. maxima wavy*	Sulawesi Maluku Papua	Yagiza Nursery
N46	*N. treubiana*	Papua	Yagiza Nursery
N47	*N. insignis*	Papua	Yagiza Nursery
N35	*N. neoguenensis*	Papua	Venom Nursery
N12	*N. reinwardtiana* x *N. eustachya*		Yagiza Nursery
N9	*(N. eustachya* x *N. reinwardtiana)* x *N. clipeata*		Yagiza Nursery
N23	*N. eustachya* x *N. beccariana*		Yagiza Nursery
N11	*N. eustachya* x *N. ampullaria*		Venom Nursery
N22	*N. ampullaria* x *N. sumatrana sibolga*		Yagiza Nursery
N1	*(N. ampullaria* x *N. globosa)* x *(N. bicalcarata* x *N. globosa)*		Venom Nursery
N2	*N. globosa* x *N. gracilis*		Venom Nursery
N3	*(N. bicalcarata* x *N. mirabilis)* x *(N. bicalcarata* x *N. globosa)*		Venom Nursery
N51	*N. ampullaria* x*N. reinwardtiana*		Yagiza Nursery
N10	*N. reinwardtiana* x *N. mirabilis*		Tulungagung Nepenthes Community
N21	*N. northiana* x *N. veitchii*		Yagiza Nursery

stems and leaves referring to Tjitrosoepomo (2018), and the observations of the pitchers referred to (Setiawan et al., 2013) which had been modified. This research was a descriptive study with a survey method to find out the types of pitcher plants (*Nepenthes* sp.), which were located at the research site. Each type of pitcher plants (*Nepenthes* sp.) was found, was morphologically identified in terms of leaves, stems, and pitchers, and was characterized (Table 2).

This study used 31 characters were analyzed by using cluster analysis and Principal Coordinates Analysis (PCoA). Cluster analysis was done by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method in Numerical Taxonomy System for personal computer (NTSYSpc) version 2.02 (Rohlf, 1998). In addition, Principal Coordinates Analysis (PCoA) was also conducted to map the distribution of Nepenthes samples into one dimensional space that was done by GenAlEx 6.5.03 (Peakall and Smouse, 2012).

RESULTS AND DISCUSSIONS

The morphological characters were analyzed to determine the relationship among the plant taxon by using the morphological properties. Morphological properties could be used to recognize and describe similarities among the Nepenthes types. The types with close genetic relationship have many similarities (Davis and Heywood, 1973). Taxonomic distance is coefficient numbers that qualitatively describe the similarity degree of the samples. The higher the percentage of similarity value, the higher the similarity level. In contrast, the smaller the percentage of similarity value, the smaller the similarity level and the greater the dissimilarity level. To observe the similarity pattern, similarity analysis was done to Nepenthes based on 31 morphological characters of 41 accessions of Nepenthes plants in Indonesia.

Group analysis with NTSYS (Numerical Taxonomy System) 2.02 by using UPGMA (Unweighted Pair Group Method With Arithmetic Averaging) based on similarity values was done to determine the genetic similarity among the individuals of the population, and the result was displayed on a dendogram. The following dendogram analysis results based on quality characters was shown in Figure 1. The similarity coefficient in 41 Nepenthes populations ranged from 0.28 to 0.65 (Figure 1). Dendrogram analysis resulted in 41 Nepenthes populations separated in similarity coefficients of 0.28 by dividing them into two major groups. The two groups were group A with similarity coefficient of 0.37 and group B with similarity coefficient of 0.30. Group A was the Nepenthes population with stemmed leaves characteristic consisting of 17 accessions,

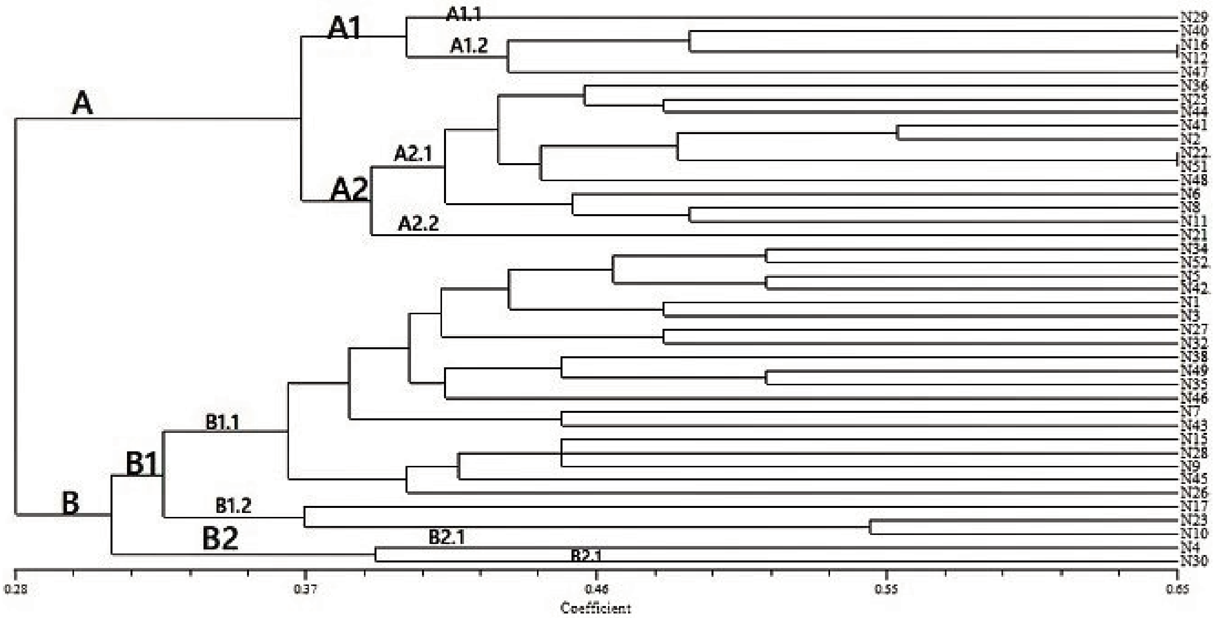

Figure 1. Dendrogram of 41 pitcher-plants' genotypes based on the qualitative characters
Description: AB: cluster; A1-A2-B1-B2: sub-cluster; A2.1-A2.2-B1.1-B2.2: sub-cluster.

Table 2. Observational variables based on the qualitative morphological characterization

Organs	No	Characters	Sub-characters
Stems	1	Color	using color chart
Leaves	2	Incomplete leaf arrangement	petioles, folius, sitting leaves, stem-hugging leaves, pseudo leaves
	3	Leaf position	alternate, opposite
	4	Leaf shape	Oval, lanceolate, obovate, elliptical, spatulate, cordate, oblanceolate, obcordate, oblong, linear, peltate, cuneate, reniform, hastate
	5	Venatio	pinnate, fingering, curved, parallel, unclear
	6	Basis folii	Acutus, acuminatus, obtusus, rotundantus, truncates, emarginatus
	7	Margo folii	flat, curved, wavy, jagged, fine-jagged, vibrating haired, serrated, fine-serrated, three-lobed, lobed
	8	Apex Folii	Acutus, acuminatus, obtusus, rotundatus, truncates, retusus, mucronatus
	9	Leaf surface	hairy, smooth
	10	Upper leaf color	using color chart
	11	Bottom leaf color	using color chart
	12	Midrib presence	yes, no
	13	Petiole presence	yes, no
	14	Petiole shape	rounded and hollow, flat and widened (winged), angular, semicircular
	15	Bone leaves color	use color chart
Pitchers	16	Pitcher shape	crock, rounded, cylindrical, funnel, waist, ovoid
	17	Waxy zone color	use color chart
	18	Degestive zone color	use color chart
	19	Inner pitcher color	use color chart
	20	Peristome shape	rounded, ovoid, rounded-jagged, inverted-heart, heart, funnel, winged-ovoid, winged-funnel
	21	Peristome color	use color chart
	22	Spur presence	yes, no
	23	Spur color	using color chart
	24	Pitcher cover color	using color chart
	25	Pitcher cover shape	rounded, ovoid, rounded-jagged, inverted-heart, heart, funnel
	26	Pitcher wing presence	yes, no
	27	Pitcher wing color	using color chart
	28	Hairy wing presence	yes, no
	29	Tendril color	using color chart
	30	Tendril surface	hairy, smooth
	31	Spur branching	single, branched

while group B had bubbly leaves characteristic consisting of 24 accessions. The dendrogram also showed that the accessions of *N. reinwardtiana* (N16) with *N. reinwardtiana* × *N. eustahcya* (N12) and *N. ampularia* × *N. sumatrana* sibolga (N22) with *N. ampularia* × *N. reinwardtiana* (N51) had the closest relationship among the 41 Nepenthes accessions. The proximity value of both was 65%. On the other side, the accession of *N. kampotiana* (N41) with *N. globosa* × *N. gracilis* (N2) and *N. eustahcya* × *N. beccariana* (N23) with *N. reinwardtiana* × *N. mirabilis* (N10) had a proximity value of 0.54. Accessions with a distant kinship were *N. ampullaria* (N4) with *N. eymae* (30) with the proximity value of 0.39.

The class differences were determined by genetic distance between genotypes in the population, which meant that genotypes in one class had narrower (similar) genetic distance. The individuals classified in one group resembled each other, while individuals in different groups had farther genetic distance than those within the same group. The more common the characters, the greater the similarity value, which indicated a closer kinship. Conversely, the more different the characters, the smaller the value of similarity, which indicated more distant kinship between the genotypes. The accessions with fewer genetic similarities were best used as parents in crossing. The greater the genetic distance (the smaller genetic similarity) between the parents, the more is the chance to obtain hybrids with a high level of heterocyst for certain characters (more than their parents or best parents) (Tatineni et al., 1996).

The increase of diversity in species occurred in apparent differences of each member of the species due to the environmental factors. The diversity of phenotypes in species could be seen from their genetic relationship with each other. The more the similarities, the closer the kinship is and vice versa. The kinship between two individuals could be measured based on the similarity level of the characters, and it was assumed that the diverse genetic arrangements led to the differences in the characters. The characters expressed by each individual were determined by their genes as the pieces of the DNA. The expressions were commonly observable in the morphological characters affected by the environment factors (Kartikaningrum et al., 2003).

Morphology is not only controlled by genetic factors but also influenced by environmental factors. Suranto (2002) stated that if environmental factors were more influential than genetic factors, plants in different places with different environmental conditions would have varied morphology, and vice versa.

Figure 1 shows the accessions of *N. reinwardtiana* (N16), *N. reinwardtiana* x *N. eustahcya* (N12), *N. eustahcya* (N15) with (*N. eustahcya* x *N. reinwardtiana*) x *N. clipeata* (N9) which were the female parents with the offspring in the same cluster and were close because of the similarity of characters found in one parent, especially with the female parent, related to the existence of cytogenic heritance pattern. The cytogenic heritance pattern states that the characteristics of the female parent are more inherited from the offspring compared to the characteristics of the male parent. This is because the genes carriers in the female parent are not only in the nucleus but also from the organelles in the cytoplasm, named mitochondria (Sunarti et al., 2018).

Principle Coordinates Analysis (PCoA) describing the relative position of each individual was conducted by using GenAlEx 6.5.03 software. PCoA is an analysis used to determine the closeness of individuals based on the similarity of characters through simplification of dimensions. PCoA was used to map individuals in the population. PCoA provides an overview of the relationships between genotypes in coordinates. Individuals with a high similarity coefficient may have been in an adjacent position, whereas individuals with a low similarity coefficient were in a remote position.

Based on the PCoA, it tended to be grouped based on the character similarity that supported and reinforced the results of grouping based on dendrograms (Figure 1). Based on Figure 2, the closeness of individuals is clearly seen by grouping together. Principle coordinate 1 shows a value of 10.58 % by separating the two populations in a large group that was the population in group I and IV with the population in group II and III, while the principle coordinate 2 with a value of 16.90% separated the two populations in group I and II with those in populationIII and IV. The individuals of pitcher plants showed an indication to form four groups. This shows that accessions located at one of the adjacent coordinates show a high level of genetic similarity (Terryana et al., 2018).

Principle Coordinate Analysis (PCoA) is very useful for analyzing genetic diversity among plant genotypes.

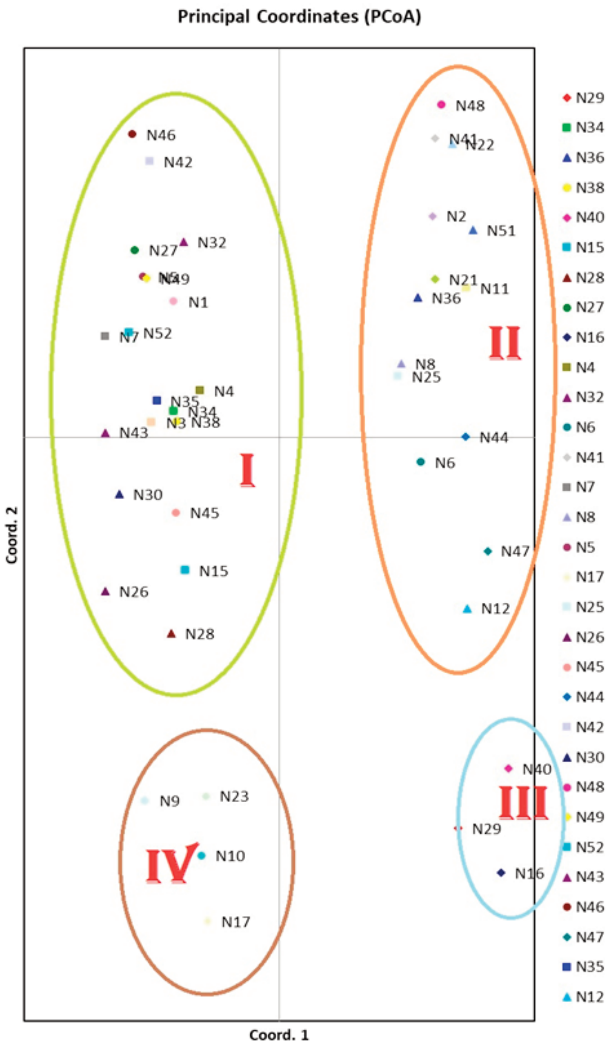

Figure 2. Principal Coordinates Analysis (PCoA) of 41 pitcher-plants' genotypes based on the qualitative characters

PCoA is able to map individuals in graphics. According to Melchinger (1993), the aggregation of individuals in the plot shows a similar genetic set of individuals. PCoA analysis using a similarity matrix shows that some individuals from one Nepenthes type mixed with individuals from other Nepenthes species populations were suspected to be from the same parent population or had a high level of phenotypic similarity.

The coordinate positions of the 41 Nepenthes population genotypes in the two-dimensional space of the PCoA results provide an opportunity to tighten plant selection activities, where genotypes with adjacent coordinates were avoided to become crossbreed parents in order to obtain prospective segregation results in certain environments (Kumar et al., 2015). The PCoA results indicate that Nepenthes populations with a high genetic diversity is significant to be used as genetic material for plant breeding because large diversity in populations is the key to succeed in plant breeding.

Accession within the same group if becomes the parents in the crossing will produce offspring with low genetic variation, while crossing among groups will produce offspring with higher genetic variation. One of the successes of plant breeding activities depends on the genetic variation. Genetic improvement of species can only be done if there are genetic differences between the parents. The genetic diversity of used germplasma is one of the important factors in succeeding a breeding program (Sujiprihati et al., 2003). Julisaniah et al. (2008) stated that the more distant the relationship between samples, the smaller the success of the crossing would be, but the possibility of obtaining superior genotype was greater if the crossing was successful. The more diverse the genetic makeup, the more likely the superior genotype would be obtained. The mating between individuals with large genetic distance or distant relationship has an effect of increasing heterozygosity. In the contrary the mating between individuals with close genetic proximity has the same effect of increasing homozygosity. This information has a good impact on the process of making superior genotypes. Mating elders with relatively high genetic variation will produce individuals with higher hererozygosity. The more distant the genetic distance between the accessions, the higher the heterocyst effect when crossed. The results of the clustering of pitcher plant species, elders, and hybrids are used as prospective elders in the more potential Nepenthes assembly. The acquired character is a prerequisite for scientific studies in crossing, propagation, and also for germplasma conservation and genetic improvement. Characterization of data is very useful not only for resource management and conversion of individual species but also for the breeders (Lokho and Kumar, 2012).

The genetic relationship in this research can be used as a reference for the plant breeding process and for differentiating the genotypes of pitcher plants. Plant breeding can produce high variations vastly when the crossing was done among species with distant relationship, so that the genetic diversity will increase in germplasma, and this condition can be exploited to obtain new variations (Martasari et al., 2009). It shows that genetic diversity within each pitcher plant populations is relatively in the same condition and is still maintained. The low genetic diversity among pitcher plant populations is strongly influenced by high gene flow. Populations with adjacent regions have sufficiently large gene flow intensity because the pollen is able to move and pollinate the plants in the closest population. In this case, the location and geographical conditions of each population helps to drive gene flow. Gene flow activities can originate from individual plants themselves such as mating systems, morphology of reproductive organs, and propagation materials through seeds.

CONCLUSIONS

The results showed that the range of the similarity coefficient of Nepenthes species was 0.28–0.65, and was divided into two major groups namely group A and B. Group A was the Nepenthes population which had petioles consisting of 17 accessions, while group B had folius consisting of 24 accessions. The greater the similarity value, the greater the similarity level between the plants. Principle Coordinates Analysis (PCoA) provided an overview of the relationships among the genotypes in coordinates. Individuals with a high similarity coefficient may have been in an adjacent position, whereas individuals with a low similarity coefficient were in a remote position. Based on the PCoA, the groups based on the similar characters supported and reinforced the grouping results on the dendrograms.

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