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GENETIC DIVERSITY OF TARO (*Colocasia* spp.) FROM KALIMANTAN ISLAND, BORNEO, INDONESIA, BASED ON RAPD MARKERS

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SUMMARY

Taro (*Colocasia esculenta* L. Schott) is a tropical plant that is grown primarily for its edible corms, which are root vegetables that are commonly known as *talas*, *kalo*, *dasheen*, and *godere*. It is widely spread and cultivated in tropical and subtropical regions, including Kalimantan Island, Indonesia. Taro is considered to be one of the oldest cultivated plants in this region and has the highest level of polymorphism. Genetic diversity in crop plants is needed to assemble germplasm for tolerance and further development against biotic and abiotic stresses. Random amplification of polymorphic DNA (RAPD) is one of the molecular methods that can be used to identify and describe genetic diversity of taro. The present research aimed to determine genetic diversity of taro accessions on the basis of RAPD molecular markers. A total of 67 taro accessions were evaluated by using 12 RAPD primers during 2016–17 at the Department of Biology, Universitas Gadjah Mada, Indonesia. Coefficient analysis based on Gower general similarity and cluster analysis were also conducted through the unweighted pair group method (UPGMA) by using MVSP version 3.1 software. Results revealed that the RAPD profiles had 105 amplified fragments with 97% polymorphism. The total fragment numbers comprised 102 with polymorphic banding patterns and three with monomorphic banding patterns. The most varied and polymorphic band was OPH-1, which had 17 banding patterns. RAPD was concluded to be capable of differentiating *C. esculenta* and *Colocasia affinis* on the basis of banding patterns. Specific bands for primers OPH-1, OPB-5, OPB-7, and OPA-17 were observed to separate *C. affinis* landraces from the accessions of other taro species that were assumed to be hybrids. The dendrogram based on UPGMA analysis showed that taro accessions from Kalimantan Island grouped into two major clusters, i.e., cluster I with one accession and cluster II with 66 accessions.

Keywords: Genetic diversity, polymorphism, RAPD markers, cluster analysis, Kalimantan Island, germplasm, *Colocasia esculenta* L., *Colocasia affinis* L.

Key findings: Taro accessions from Kalimantan Island, Indonesia, revealed the highest polymorphism. RAPD markers can be used to differentiate the genetic diversity of the

accessions of different species of taro. The two types of taro i.e., *C. esculenta* and *C. affinis*, could be differentiated on the basis of some specific DNA bands.

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INTRODUCTION

Borneo, a giant and rugged island, is the third largest island in the world and the largest in Asia; it is principally administered by Indonesia and is known as Kalimantan Island (Galappathie *et al*., 2014). This island has the highest diversity of aroid plants (Ridley, 1905). Taro (*Colocasia esculenta* L. Schott) is one of the crop plants belonging to the Araceae (Aroid) family. This plant is widely spread and cultivated in tropical and subtropical regions, including Kalimantan Island. For decades, communities in Kalimantan have recognized the taro plant for as the oldest food crop that is cultivated along with other fruit plants (MacKinnon *et al*., 2000).

Taro is a very ancient aroid plant with high levels of polymorphism and plasticity and extensive genetic variation (Matthews, 2010). Although taro is an important food crop, very little research has been carried out on its diversity, and particularly on the exploitation of existing cultivated landraces, wild types, and improved cultivars in different regions, such as India, Southeast Asia, South China, Melanesia, and Australia (Quero-Garcia *et al*., 2010; Bhattacharjee *et al*., 2014). Given that *C. esculenta* is reported to display antidiabetic, anti-inflammatory, antioxidant, and anticancer activities, it has good prospects for future development. The great diversity of aroid and taro species in Indonesia, especially that of species in Kalimantan, has not been supported by genetic information, particularly information on genetic variability (Lebot *et al*., 2010). Extensive research on the diversity and further development of taro and the identification of taro cultivars with tolerance to biotic and abiotic stresses is needed (Invanvic and Lebot 1999; Sharma *et al*., 2008), especially taro leaf blight (Nath *et al*., 2015).

Molecular techniques are very helpful for identifying and exploiting genetic diversity. The characterization of germplasm by using molecular markers can produce information regarding the genetic relationship between wild and cultivated taro plants. In addition, it can be used as basic information to develop the genetic diversity of taro genotypes further. This information also provides the basis to plant breeders and growers for the cultivation of taro types that are desired by the market and possessing resistance to environmental factors (Sharma *et al*., 2008; Das and Das, 2014). Molecular analysis is particularly useful for the identification of genotypes and variability and for phylogenetic and genetic mapping (Yu and Nguyen, 1994). Molecular techniques can help understand the relationship between different taxonomic groups.

As a crop plant, taro has morphological and agronomic character variation. Morphological characters are mainly based on leaves, roots, and corms, with leaf organs, particularly the petiole, exhibiting the highest variation. Color variation is also very important in the identification of species and their different cultivars (Oktavianingsih *et al*., 2019; Purnomo *et al*., 2020). A thorough study of the genetic diversity, variation, and wild relatives of taro is therefore needed for the development of taro as an alternative food crop in the future. The usefulness of DNA markers, such as RAPDs, in characterizing and assessing the genetic diversity of taro germplasm is hereby established. RAPD markers are also useful

for distinguishing taro accessions on the basis of origin country and ploidy level. Research on genetic diversity in taro by using RAPD markers has been conducted in Indonesia. Such research classified Indonesian taro into two major groups but did not fully include the taro accessions from Kalimantan Island (Hartati *et al*., 2001). On the basis of the above facts, the present research was conducted to study the genetic diversity of the taro accessions from Kalimantan Island by using RAPD markers.

MATERIALS AND METHODS

Plant materials and procedure

Sixty-seven taro accessions were collected from various habitats in all provinces of Kalimantan Island, Indonesia. Sample selection for RAPD was performed through phenotypic variation and cluster analysis based on morphological characters (Oktavianingsih *et al.*, 2019) (Table 1). The said study was performed during 2016–17 at the Department of Biology, Universitas Gadjah Mada, Indonesia.

DNA isolation and polymerase chain reaction amplification

DNA isolation was performed by using the modified C-TAB method in reference to Sharma *et al*. (2008). DNA amplification by using the polymerase chain reaction (PCR)–RAPD technique with 12 primers from integrated DNA Technologies USA (Table 2). The PCR mixture had a total volume of 25 µl and contained 2.5 µl of 10 ng of template DNA, 20 µl of 1× Master mix (KAPA HotStart), and 2.5 µl of 50 pmol primers.

DNA amplification was performed by using a PCR machine with Thermocycler under the following conditions: initial denaturation for 2 min at 94 °C, denaturation for 0.5 min at 94 °C, annealing for 1 min at 37 °C, extension for 2 min at 72 °C, 35×, and continued with a final extension cycle for 8 min at 72 °C. The amplified DNA was then electrophoresed on 1.2% agarose gel containing 0.5 mg/ml Fluorasave at 50 V for 55 min. The amplified product results were observed on 1.5% agarose gel containing 0.5 mg/ml Fluorasave with a UV trans-illuminator. The band (profile) of DNA was then photographed. Twelve primers were used for PCR–RAPD amplification (Table 2).

Data analysis

Molecular data analysis was based on the presence or absence of the band generated with each primer. Any DNA band present in electrophoresis was measured by using a linear regression equation. The focus was converted into a binary data matrix by giving a score of 1 if a DNA band was present and 0 if a DNA band was absent. The binary data matrix was then derived into a genetic distance matrix with a Gower similarity coefficient. Cluster analysis and dendrograms were made by using Unweighted Pair Group Method (UPGMA) with MVSP version 3.1 computer software program.

RESULTS

RAPD band amplification

All the selected primers could produce polymorphic amplification products (Table 3). Amplification with the 12 RAPD primers yielded 105 RAPD bands that consisted of 102 and three polymorphic and monomorphic bands, respectively. The bands varied from 3 (OPW-12) to 17 (OPH-1). The polymorphism of taro from Kalimantan island reached 97% based on 102 RAPD polymorphic bands. The highest level of polymorphism shown by the taro accessions from Kalimantan Island indicated high genetic diversity. The primers, nucleotide sequences, numbers, sizes, and nature of the RAPD band (monomorphic and polymorphic) are shown in Table 3.

Table 1. The Taro accessions with species names and their origin used in molecular studies.

No.	Accessions	Species	Local Cultivar	Origin Accessions
1.	KT-1	*C. esculenta*	Keladi Gunung	Samarinda-East Kalimantan
2.	KT-2	*C. esculenta*	Keladi Sulur	Samarinda-East Kalimantan
3.	KT-3	*C. esculenta*	Keladi Lais	Samarinda- East Kalimantan
4.	KT-4	*C. esculenta*	Keladi Sayur	Kutai Timur-East Kalimantan
5.	KT-6	*C. esculenta*	Keladi Hitam	Bontang-East Kalimantan
6.	KT-7	*C. esculenta*	Keladi Hitam	Kutai Timur-East Kalimantan
7.	KT-9	*C. esculenta*	Keladi Liar	Kutai Kertanegara-East Kalimantan
8.	KT-10	*C. esculenta*	Keladi Liar	Kutai Kertanegara-East Kalimantan
9.	KT-13	*C. esculenta*	Talas Malaysia	Samarinda-East Kalimantan
10.	KT-14	*C. esculenta*	Talas Liar	Samarinda-East Kalimantan
11.	KT-15	*C. esculenta*	Talas Jahe	Samarinda-East Kalimantan
12.	KT-17	*C. esculenta*	Keladi	Samarinda-East Kalimantan
13.	KT-20	*C. esculenta*	Keladi Liar	Samarinda-East Kalimantan
14.	KT-22	*C. esculenta*	Keladi Gatal	Kutai Kertanegara-East Kalimantan
15.	KT-24	*C. esculenta*	Keladi Sayur	Kutai Barat-East Kalimantan
16.	KT-25	*C. esculenta*	Keladi Gunung	Kutai Barat-East Kalimantan
17.	KT-31	*C. esculenta*	Keladi Akar	Samarinda-East Kalimantan
18.	KU-36	*C. esculenta*	Talas Malaysia	Tarakan-North Kalimantan
19.	KU-38	*C. esculenta*	Talas Hitam	Tarakan-North Kalimantan
20.	KU-39	*C. esculenta*	Talas Ungu	Tarakan-North Kalimantan
21.	KS-41	*C. esculenta*	Keladi Putih	Kab.Tanah Laut-South Kalimantan
22.	KS-43	*C. esculenta*	Keladi Bentul	Kab.Tanah Laut-South Kalimantan
23.	KS-44	*C. esculenta*	Keladi Hutan	Martapura-South Kalimantan
24.	KS-46	*C. esculenta*	Keladi Liar	Kab.Banjar-South Kalimantan
25.	KS-49	*C. esculenta*	Keladi Liar	Banjarmasin-South Kalimantan
26.	KS-51	*C. affinis*	Keladi Hias	Kab.Banjar-South Kalimantan
27.	KS-52	*C. esculenta*	Talas Telur	Kab.Tapin-South Kalimantan
28.	KS-57	*C. esculenta*	Talas Liar	Kab.Hulu Sungai Selatan-South Kalimantan
29.	KS-58	*C. esculenta*	Keladi Hitam	Kab.Hulu Sungai Selatan-South Kalimantan
30.	KS-59	*C. esculenta*	Keladi Putih	Kab.Hulu Sungai Selatan-South Kalimantan
31.	KS-60	*C. affinis*	Keladi Liar	Kab.Hulu Sungai Selatan-South Kalimantan
32.	KS-61	*C. affinis*	Keladi Liar	Kab.Hulu Sungai Selatan-South Kalimantan
33.	KS-65	*C. esculenta*	Keladi Hitam	Kab.Balangan-South Kalimantan
34.	KH-70	*C. esculenta*	Keladi Hitam	Kab.Katingan-Central Kalimantan
35.	KH-73	*C. esculenta*	Keladi Hitam	Sampit-Central Kalimantan
36.	KH-74	*C. esculenta*	Keladi Putih	Sampit-Central Kalimantan
37.	KH-75	*C. esculenta*	Keladi Liar	Palangkaraya-Central Kalimantan
38.	KH-76	*C. esculenta*	Keladi Sayur	Palangkaraya-Central Kalimantan
39.	KH-77	*C. esculenta*	KujangBawa’	Kab.Pulang Pisau-Central Kalimantan
40.	KH-78	*C. esculenta*	Kujang Enyuh	Kab.Pulang Pisau-Central Kalimantan
41.	KH-79	*C. esculenta*	Kujang Gahuri	Kab.Pulang Pisau-Central Kalimantan
42.	KH-84	*C. affinis*	Keladi Liar	Kab.Kapuas-Central Kalimantan
43.	KH-86	*C. esculenta*	Keladi Habang	Kab.Kapuas-Central Kalimantan
44.	KB-88	*C. esculenta*	Keladi Liar	Kab.Kubu Raya-West Kalimantan
45.	KB-89	*C. esculenta*	Keladi Liar	Kab.Kubu Raya-West Kalimantan
46.	KB-90	*Colocasia* sp.	Keladi Liar	Kab.Kubu Raya-West Kalimantan
47.	KB-91	*C. esculenta*	Keladi Tikus	Pontianak-West Kalimantan
48.	KB-95	*C. esculenta*	Keladi Hitam	Pontianak-West Kalimantan
49.	KB-96	*C. esculenta*	Keladi Kelapa	Pontianak-West Kalimantan
50.	KB-98	*C. esculenta*	Keladi Kelapa	Mempawah-West Kalimantan
51.	KB-101	*C. esculenta*	Keladi Liar	Kab.Mempawah-West Kalimantan
52.	KB-102	*C. esculenta*	Keladi	Kab.Mempawah-West Kalimantan
53.	KB-104	*C. esculenta*	Keladi Bangkok	Mempawah-West Kalimantan
54.	KB-105	*Colocasia* sp.	Keladi Liar	Mempawah-West Kalimantan
55.	KB-106	*C. esculenta*	Keladi Liar	Kab.Landak-West Kalimantan
56.	KB-107	*C. esculenta*	Talas Merah	Kab.Landak-West Kalimantan
57.	KB-108	*C. esculenta*	Talas Putih	Kab.Landak-West Kalimantan
58.	KB-109	*C. esculenta*	Talas Minyak	Kab.Landak-West Kalimantan
59.	KB-111	*C. esculenta*	Keladi Hitam	Kab.Sanggau-West Kalimantan
60.	KB-112	*C. esculenta*	Talas Malaysia	Kab.Sanggau-West Kalimantan
61.	KB-113	*C. esculenta*	Keladi Cina	Kab.Sanggau-West Kalimantan
62.	KB-114	*C. esculenta*	Keladi Mei	Kab.Sanggau-West Kalimantan
63.	KB-115	*C. esculenta*	Keladi Mei Hitam	Kab.Sanggau-West Kalimantan
64.	KB-116	*C. esculenta*	Keladi Udang	Kab.Sanggau-West Kalimantan
65.	KB-118	*C. esculenta*	Keladi Madura	Kab.Kubu Raya-West Kalimantan
66.	KB-119	*C. esculenta*	Keladi Sayur	Kab.Kubu Raya-West Kalimantan
67.	KB-123	*C. esculenta*	Keladi Tikus	Kab.Kubu Raya-West Kalimantan

Table 2. Primers and their nucleotide base sequences used in RAPD-based amplification.

No.	Primer name	Primer sequence (5'–3')	G + C content (%)
1.	OPB-05	TCAGCGAGGT	60
2.	OPB-07	GGTGACGCAG	70
3.	OPB-15	GGAGGGTGTT	60
4.	OPB-20	GGACCCTTAC	60
5.	OPW-08	GACTGCCTCT	60
6.	OPW-10	TCGCATCCCT	60
7.	OPW-12	TGGGCAGAAG	60
8.	OPW-15	ACACCGGAAC	60
9.	OPH-01	AATCGGGCTG	60
10.	OPA-17	GACCGCTTGT	60
11.	OPG-13	CTCTCCGCCA	70
12.	OPK-19	CACAGGCGGA	70

Sources : Hartati et al. (2001), Ochiai et al. (2001), Prana et al. (2010).

Table 3. Primers, nucleotide sequences, and number of RAPD bands.

No.	Primers	Nucleotide sequence (5'–3')	RAPD bands	Polymorphic bands	Monomorphic bands	RAPD band size (base pairs)
1.	OPB-05	TCAGCGAGGT	9	9	0	300–1200
2.	OPB-07	GGTGACGCAG	12	12	0	300–1200
3.	OPB-15	GGAGGGTGTT	8	8	0	300–1200
4.	OPB-20	GGACCCTTAC	11	10	1	200–1300
5.	OPW-08	GACTGCCTCT	9	9	0	200–1300
6.	OPW-10	TCGCATCCCT	5	5	0	300–1300
7.	OPW-12	TGGGCAGAAG	3	2	1	600–1500
8.	OPW-15	ACACCGGAAC	11	11	0	150–1700
9.	OPH-01	AATCGGGCTG	17	17	0	250–1700
10.	OPA-17	GACCGCTTGT	7	7	0	250–1350
11.	OPG-13	CTCTCCGCCA	6	5	1	200–1100
12.	OPK-19	CACAGGCGGA	7	7	0	200–1200

RAPD primer information and band size

A very informative primer was indicated and confirmed by having amplification results with more than five bands. The most varied and polymorphic primer was OPH-1 with 100% and size ranging from 250 bp to 1700 bp (Figure 1). Primers featuring polymorphic bands were found with primers OPB-07 (12 DNA bands), OPB-20 (10 DNA bands), OPH-01 (17 DNA bands), and OPW-15 (11 DNA bands) with band sizes ranging from 200 bp to 1700 bp. The primers that exhibited monomorphic bands were OPB-20 (one DNA band), OPW-12 (one DNA band), and OPG-13 (one DNA band) with sizes of 200 bp to 1500 bp (Figure 2). The primers that produced polymorphic bands without monomorphic bands were OPB-5 (nine DNA bands), OPB-7 (12 DNA bands), OPB-15 (eight DNA bands), OPW-8 (nine DNA bands) OPW-10 (five DNA bands), OPW-15 (11 DNA bands), OPH-01 (17 DNA bands), OPA-17 (seven DNA bands), and OPK-19 (seven DNA bands).

This study, the DNA band sizes varied from 150 bp to 1700 bp. The shortest bands were found with primers OPW-15, whereas the longest bands were recorded for OPW-15 and OPH-01. The current findings were slightly different from the results of earlier studies on the genetic diversity of taro accessions wherein the band sizes varied from 300 bp to 1500 bp (Hartati *et al*., 2001).

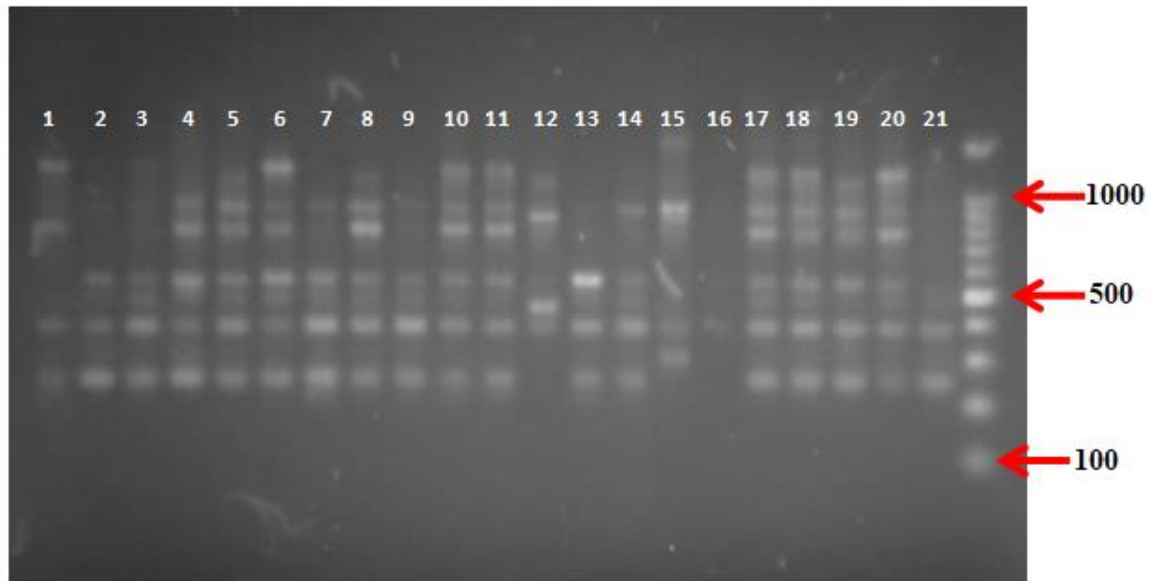

Figure 1. RAPD primer profiles showing the most polymorphic bands (OPH-1) 1. KT-2, 2. KT-7, 3. KT-9, 4. KT-14, 5. KT-17, 6. KT-20, 7. KT-22, 8. KT-24, 9. KU-39, 10. KS-43, 11. KS-44, 12. KS-49, 13. KS-57, 14. KS-58, 15. KS-61, 16. KS-65, 17. KS-77, 18. KS-79, 19. KS-86, 20. KB-95, and 21. KB-102.

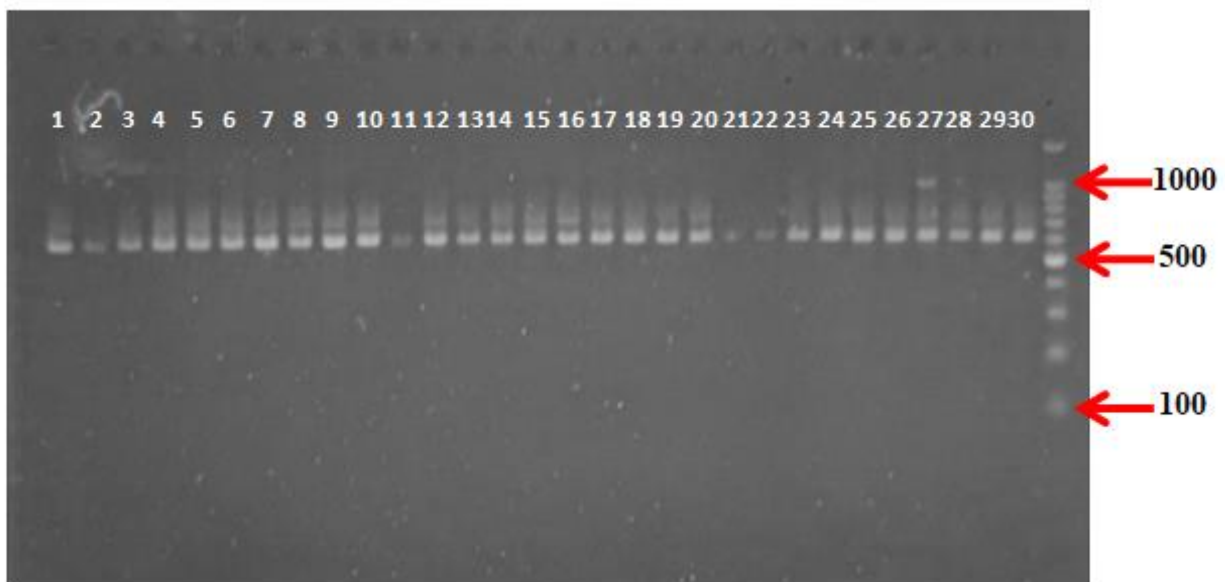

Figure 2. RAPD primer profile showing the monomorphic bands (OPW-12) 1. KT-14, 2. KH-73, 3. KH-74, 4. KH-76, 5. KH-75, 6. KH-77, 7. KH-78, 8. KH-79, 9. KH-86, 10. KB-88, 11. KB-89, 12. KB-90, 13. KB-9, 14. KB-95, 15. KB-96, 16. KB-98, 17. KB-102, 18. KB-104, 19. KB-106, 20. KB-107, 21. KB-108, 22. KB-109, 23. KB-111, 24. KB-112, 25. KB-113, 26. KB-114, 27. KB-116, 28. KB-118, 29. KB-119, and 30. KB-123.

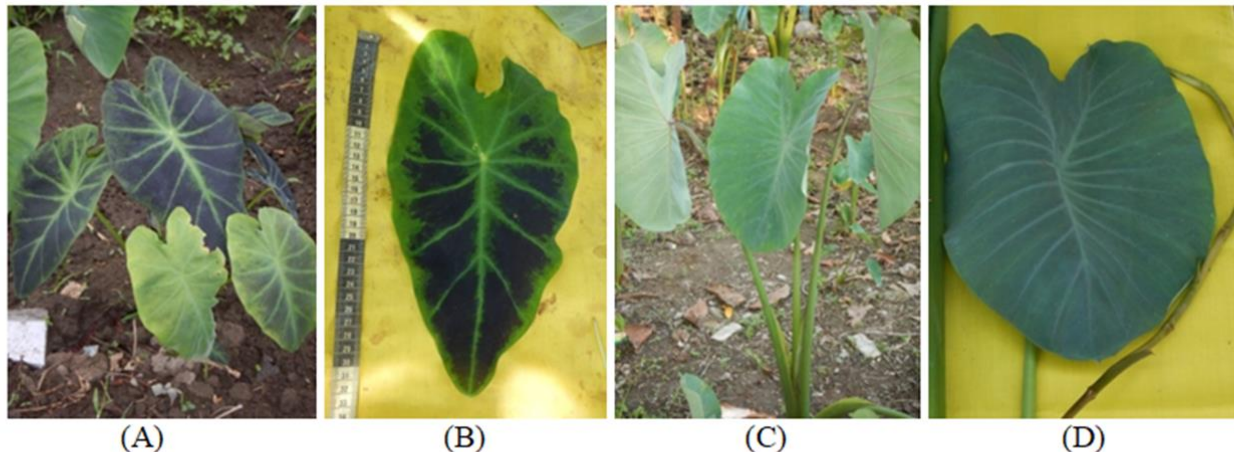

Figure 3. Taro leaves A and B: *Colocasia affinis* has blackish-purple patches on its leaf surface, C and D *Colocasia esculenta* lacks blackish-purple patches on its leaf surface.

Figure 4. Specific primers that can separate *Colocasia affinis* from other taro species (OPB-07). 1. KT-2. KT-2, 3. KT-3, 4. KT-4, 5. KT-6, 6. KT-7, 7. KT-9, 8. KT-10, 9. KT-13, 10. KT-14, 11. KT-15, 12. KT-17, 13. KT-20, 14. KT-22, 15. KT-24, 16. KT-25, 17. KT-31, 18. KU-36, 19. KU-38, 20. KU-39, 21. KS-41, 22. KS-43, 23. KS-44, 24. KT-46, 25. KS-49, 26. KS-51, 27. KS-52, 28. KS-57, 29. KS-58, 30. KS-59, 31. KS-60, 32. KS-61, and 33. KS-65.

The obtained RAPD bands were not capable of separating the taro groups on the basis of location. Instead, the differentiation was made on the basis of different species, i.e., *C. esculenta* and *Colocasia affinis*. The present results are noteworthy given that no previous report based on the RAPD method could classify taro accessions belonging to different species. On the basis of morphological characters, the species *C. affinis* remained in the same large group with *C. esculenta* with a similarity coefficient of 0.65 (Oktavianingsih, 2019). The present results revealed that both taro species were also morphologically different,

especially in terms of leaf surface color (Figure 3). The species *C. affinis* has blackish-purple patches on leaf surfaces. Matthews (2014) stated that *C. affinis* might likely be the result of interspecies hybridization that resulted in morphological variations with their ancestors.

Some other specific bands could be used to separate the accessions of *C. affinis* from other taro accessions. Special bands for *C. affinis* were found with the primers OPH-1, OPB-5, OPA-17, and OPB-7 with sizes of 600, 700, and 900 bp, respectively (Figure 4). *C. affinis* accessions were derived from different regions, i.e., KS-51, KS-60, and KS-61 originated from South Kalimantan Province, whereas KT-84 was related to species from Central Kalimantan Province.

Cluster analysis

RAPD cluster analysis showed that taro accessions from Kalimantan Island had similarity indexes ranging from 0.688 (68.8%) to 0.943 (94.3%). Taro accessions from Kalimantan Island were divided into two large clusters i.e., cluster I consisted of one accession, whereas cluster II had 66 accessions. Cluster I (A) consisted of one member, namely, accession KB-101 (*C. esculenta* 'Keladi Hutan'). It was separated from other clusters with a similarity coefficient index of 0.688 on the basis of the primers OPH-01 (0.25, 0.5, 0.66, 0.7, and 1.1 kb), OPB-07 (0.4, 0.5, 0.7, and 1.1 kb), OPB-05 (0.3, 0.4, 0.6, 0.8, and 1.1 kb), OPB-15 (0.3, 0.5, 0.8, and 0.9 kb), OPB-20 (0.4 and 0.5 kb), OPW-08 (0.3 and 1.2 kb), OPW-15 (1.5, 0.2, 1.2, and 1.5 kb), OPW-10 (0.5 and 1 kb), and OPA-17 (0.25, 0.35, 0.5, and 1.25 kb). This taro accession grows on the edge of the forest in Mempawah, West Kalimantan Province, Indonesia.

Cluster II (B) comprised 66 taro accessions that were divided into five subgroups related to different species and their cultivars with a similarity coefficient index of 0.712 (71.2%). Subgroup B1.a had only one member accession, namely, KS-65 (*C. esculenta* 'Keladi Hitam'), which is used by society as a staple food for patients with diabetes. This cultivar is planted and cultivated in rice fields by communities, like the community in South Kalimantan Province. This subgroup was separated from other accessions on the basis of primer bands, i.e., OPH-01 (0.25, 0.6, and 0.8 kb), OPB-07 (0.3 and0.35 kb), OPB-05 (0.4 kb), OPB-15 (0.6 kb), OPB-20 (0.2 kb), OPW-8 (0.2, 0.8, 1, and 1.3 kb), OPW-15 (0.2 and 1.5 kb), OPW-10 (1 kb), OPA-17 (0.6 and 1.35 kb), and OPK-19 (0.4 kb). Subgroup B1.b consisted of six accessions that were grouped with a similarity coefficient of 0.74 with the bands of primers OPH-01 (0.35, 0.8, 0. 95, 1.2, 1.5, and 1.7 kb), OPB-07 (0.3, 3.5, 0.8, 1.1, 1.2, and 1.3 kb), OPB-05 (0.35, 0.9, and 1 kb), OPB-15 (1 kb), OPB-20 (0.3, 0.8, 1, 1.2, and 1.3 kb), OPW-08 (0.2, 0.3, 0.4, 0.6, 0.9, and 1.3 kb), OPW-15 (0.3, 1.3, 1.5, and 1.7 kb), OPW-12 (1.1 and 1.5 kb), OPW-10 (0.3 and 1 kb), OPA-17 (0.5, 0.6, 1.25, and 1.35 kb), OPG-13 (0.3 kb, 0.4, 0.8, 1, and 1.1 kb), and OPK-19 (0.6, 0.7, 1.1, and 1.2 kb). All these subgroups belonged to species *C. esculenta* but differed in terms of morphological characters and cultivar names. Accessions belonging to this subgroup were separated into four taro accessions that were collected from West Kalimantan, one accession from Central Kalimantan, and one taro accession from South Kalimantan Province.

Subgroup B2.a consisted of four accessions, i.e., KB-118, KB-116, KB-111, and KB-106. Theses accessions were grouped with *C. esculenta* but were different from local taro cultivars. This group of taro genotypes was collected from West Kalimantan Province but originated from a different region. This group clustered on the basis of the bands of primers OPH-01 (0.3, 0.35, 0.6, 0.9, 0.95, 1.1, 1.3, 1.5, and 1.7 kb), OPB-07 (0.35, 0.5, 0.6, 0.8, 1, 1.1, 1.2, and 1.3 kb), OPB-05 (0.35, 1, 1.25, and 1.35 kb), OPB-15 (0.6, 0.8, and 1 kb), OPB-20 (0.2, 0.3, 0.4, 0.5, 0.8, 1, 1.1, and 1.2 kb), OPW-08 (0.3, 0.4, 0.6, 0.8, 0.9, 1.2, and 1.3 kb), OPW-15 (0.7, 0.8, 1, 1.2, and 1.7

Figure 5. Dendrogram based on RAPD markers showing the separation of taro accessions from Kalimantan Island into two large clusters.

kb), OPW-12 (1.1 kb), OPW-10 (0.3, 0.4, 0.5, and 1.3 kb), OPA-17 (1.25 and 1.35 kb), OPG-13 (0.3, 0.4, 0.8, 1, and 1.1 kb), and OPK-19 (0.2, 0.6, 0.7, 0.9, 1.1, and 1.2 kb).

Subgroup B2.b1 was related to *C. affinis* with a similarity coefficient of 0.799. The dendrogram results showed that this species merged into one separate subgroup on the basis of the bands of primers OPH-01 (0.25, 0.3, 0.5, 0.6, 0.7, 0.9, 1.1, 1.3, 1.5, and 1.7 kb), OPB-07 (0.35, 0.7, 0.8, 0.9, 1, 1.1, 1.2, and 1.3 kb), OPB-05 (0.35, 0.6, 0.8, 0.9, 1, 1.1, and 1.2 kb), OPB-15 (0.3, 0.5, 0.6, 0.8, 0.9, 1, 1.1, and 1.2 kb), OPB-20 (0.2, 0.3, 0.5, 0.8, 0.9, 1, and 1.1 kb), OPW-8 (0.6, 0.8, 0.9, 1.2, and 1.3 kb), OPW-15 (0.2, 0.3, 1, 1.2, 1.3, and 1.7 kb), OPW-12 (1.1 and 1.5 kb), OPW-10 (0.5, 1, and 1.3 kb), OPA-17 (0.35 and 0.5 kb), OPG-13 (0.2, 0.3, 0.8, and 1 kb), and OPK-19 (0.2, 0.4, 0.6, 0.7, and 0.9 kb). Specific primers that separated this group were OPH-1 (0.3 kb), OPB-5 (1.35 kb), OPB-7 (0.6, 0.9 kb), and OPA-17 (0.5 kb). These taro accessions had specific leaf surface morphological characteristics, such as dark purple or blackish purple patches. These species are generally used as ornamental plants by the community because of their leaf surface color. The subgroup B2.b2 belonged to cluster II with a similarity coefficient of 0.729 (72.9%) and is generally a species of *C. esculenta.* This subgroup consisted of 50 taro accessions (Figure 5). On the basis of their similarity indexes, which ranged from 0.688 (68.8%) to 0.943 (94.3%), all the taro accessions could be assumed to belong to *C. esculenta*. However, the results also supported the possibility that these accessions belonged to the hybrid species *C. affinis*.

DISCUSSION

RAPD molecular analysis revealed that taro accessions from Kalimantan island had high polymorphism (97%). These findings were supported by an early study on the molecular characterization of taro (*C. esculenta*) by using RAPD markers that provided the same observations (Irwin *et al*., 1998). The RAPD markers in this study showed the high genetic diversity of taro accessions from Indonesia as was also supported by a dendrogram based on morphological characteristics, which revealed that taro accessions from Kalimantan Island, Indonesia (Oktavianingsih, 2019), and Java, Indonesia, had the highest genetic diversity (Purnomo *et al*., 2020).

The dendrogram results showed two large clusters with six groups of taro cultivars and types. Given that the similarity indexes ranged from 0.688 (68.8%) to 0.943 (94.3%), all the taro accessions could be assumed to consist of one species and supported the possibility that they were members of the hybrid species *C. affinis*. Marsolais *et al*. (1993), by using RAPD molecular analysis, obtained similarity values for lilac (*Syringa vulgaris* L.) that ranged from 0.61% to 0.99% and were considered as species limitation.

In general, cluster analysis based on molecular characters and RAPD markers did not support the clear separation of taro accessions. Hartati *et al*. (2001) also revealed the absence of equivalence between morphological characters and RAPD molecular markers. Singh *et al*. (2012) reported the a lack of perfect similarity between morphological and molecular characters. However, RAPD markers could classify the taro species *C. affinis* into its own cluster on the basis of the presence of a specific band. *C. affinis* was likely the result of interspecies hybridization. These results were supported by Long and Liu (2001), who suggested the possibility that the hybrid taro *Colocasia* could occur in nature. Interspecies hybridization is crucial for evolution in that it might cause polymorphism in taro (Matthews, 2014).

RAPD molecular analysis was found to be best suited for detecting genetic differences within *C. esculenta* L. and among its related species (Ochiai *et al*., 2001; Prana *et al*., 2010). Hanum (2013) reported that in their taxonomy study,

specific DNA bands might be considered as either analytic or diagnostic in nature. Analytic nature (diagnostic) indicates that a character could be applied to identify, characterize, and restrict taxa with a specific and limited existence to differentiate a taxon from its close relatives (Davis and Heywood, 1973). Singh (2012) reported that molecular markers might strengthen the solution of the problem of genetic diversity and provide complementary information on the collection, management, and development of germplasm. RAPD molecular markers have been the choice of several researchers who worked on *C. esculenta* L. and tried to produce high levels of polymorphism (Singh *et al*., 2012).

The dendrograms based on molecular markers did not show cluster separation on the basis of the spread and distribution areas of taro in Kalimantan Island, Indonesia. This situation was supported by the opinion of Sharma *et al*. (2008) and Prana *et al*. (2010), suggesting that isozymic and RAPD molecular markers could indicate the absence of correlation between cluster formation and geographic regions. The variation in the RAPD markers of taro under natural selection showed low correlations between populations separated by geographical distances.

CONCLUSIONS

RAPD markers were concluded to be helpful for finding the highest level of polymorphism in taro species. The polymorphism of taro from Kalimantan Island, Indonesia, reached 97%. Thus, the highest level of genetic diversity in taro accessions was demonstrated indirectly. On the basis of cluster analysis, the taro accessions from Kalimantan Island were classified into two major clusters with six subgroups. The DNA bands could differentiate the two types of taro, i.e., *C. esculenta* and *C. affinis*, on the basis of some specific bands.

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