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GENETIC DIVERSITY IN TARO (*COLOCASIA ESCULENTA* L. SCHOTT) GERMPLASM PROCURED FROM SEVEN PROVINCES OF INDONESIA

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SUMMARY

Indonesia has a great diversity of taro *(Colocasia esculenta* L. Schott) which is cultivated and used as a non-rice source of carbohydrates. Taro could be processed for various foods, including baby food, as well as raw material for cosmetic and medical industries. Studies on genetic diversity could be carried out by using molecular markers, in which the ISSR (inter simple sequence repeat) markers technique is very important. The aim of this study was to assemble the information regarding genetic diversity in the collected germplasm of taro comprising 50 genotypes (42 cultivars and eight accessions). PCR recapitulation showed that 17 (56.7%) out of 30 ISSR primers were used to have high level of polymorphism. However, eight ISSR markers (HB 8, HB 10, HB 12, HB 14, 844A, 844B, 814, and UBC 15) by having PIC scores of 0.71 to 0.73, could be effectively used to distinguish the taro cultivars and accessions. The 218 alleles were recorded in total, with an average of 12.82. Each taro cultivar has different number of alleles ranging from 3 to 25. The PCR optimization with various aspects was required in order to obtain the optimal results. The closest genetic distance with 98% similarity index was found at Bentul cultivar (with cultivar numbers 10 and 11) and suggested as sister lines. On the other hand, the lowest similarity index (65%) was found between cultivar numbers 46 and 34 belongs to Bentul and Bitik, respectively. Taro cultivars and accessions from West Java province revealed the highest genetic diversity as compared to other provinces. Cluster analysis showed that provinces having origin of taro germplasm were not necessarily related with genetic clustering. Results also concluded that some taro cultivars and accessions with the same name were not always found in the same cluster, suggesting that cultivars and accessions collected from different regions have different genetic background.

Key words: *Colocasia esculenta* L., taro germplasm, genetic diversity, ISSR markers, cluster analysis

Key findings: Genetic diversity was observed in Indonesian Taro (*Colocasia esculenta* L.) cultivars and accessions based on ISSR markers.

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INTRODUCTION

Colocasia esculenta L. Schott belongs to the family of *Araceae,* known as taro and spread worldwide. The largest production area of taro found in Africa, South East Asia, and subtropical countries, specifically in China, Japan, and Pacific Islands (Lebot *et al*., 2004). In addition to food source, taro is also used as a raw material in traditional medicines in China (Li *et al*., 2004). Indonesia is one of mega-biodiversity countries of plants species, including taro. Similar to other countries, taro has been cultivated and used as a non-rice source of carbohydrates with sufficient nutritive contents in Indonesia (http://seafast.ipb.ac.id). Taro could be processed as various foods, including baby food and as raw material for different cosmetics and medicines. As a food, taro could be processed by roasting, toasting, boiling, and processing into fresh or fermented pasta, flour, drink, and crispy chips. Besides the tuber, taro leaves could be consumed as a vegetable because of having highest source of vitamin, mineral, secondary metabolites, and fiber.

Taro is one of the important economic plants, in which its agronomic characters need to be developed and improved. Plant genetic improvement has been applied to obtain superior characteristics of the plant, such as high productivity, resistance to biotic and abiotic stresses, starch content and nutritional value. Generally, taro propagation was developed by vegetative organ, using the roots. Meanwhile, taro cultivar improvement was carried out through crossing which takes from six to eight months for blooming after planting (Prana, 2007). To develop new cultivar, rich plant germplasm is needed which can bring the desired characteristics. Therefore, taro germplasm with greater genetic diversity and broad genetic distances is needed to obtain new improved cultivars with good genetic yield potential.

Indonesia has a great diversity of taro ranging from wild to cultivated and commercial taro (Prana, 2007). Study on genetic diversity could be conducted both in traditional and modern ways. Traditionally, morphological diversity analysis based on plant phenotypic traits has been conducted for a long time (Vinutha *et al*., 2015). However, the morphological observation which basically represents only a small part of plant genome, and is easily influenced by environmental factors (Golabadi *et al*., 2012). Therefore, a modern diversity analysis using molecular markers is considered efficient as it has been developed and applied widely. Molecular markers analysis is divided into hybridization based methods such as RFLP (restriction fragment length polymorphism) and DNA PCR (polymerase chain reaction). There

are five techniques of PCR based method, namely RAPD (random amplified polymorphism DNA), AFLP (amplified fragment length polymorphism), SNP (single nucleotide polymorphism), SSR (simple sequence repeat), and ISSR (inter simple sequence repeat) (Agrawal *et al*., 2008, Poyraz, 2016). Moreover, the analysis could be done in the mixed form of morphological markers correlated with modern markers (Singh *et al.,* 2008; Singh *et al*., 2012; Fang *et al.,* 2018; Sanchez-Velazquez and Ramos-Diaz, 2018). In addition to molecular based markers, diversity analysis can also be conducted by applying other methods, such as *isoenzyme*, secondary metabolite diversity, sequencing, and cytology (Damares *et al*., 1995; Mathews, 2004; Nauheimer *et al.,* 2012; Munoz-Cuervo *et al.,* 2016; Senavangse *et al*., 2018). These analyses not only identify the genetic diversity, but also lead to investigate desired traits (Monfared *et al.,* 2018). A measured genetic diversity could be used for further improvement and future conservation of the genetic resources (Devi, 2012).

Genetic diversity was studied in Asian and Pacific taro through AFLP technique using diploid and triploid cultivars and accessions as well as wild germplasm (Kreike *et al*., 2004). The cultivars and accessions were collected from Thailand, Malaysia, Vietnam, Indonesia, Philippines, Papua New Guinea and Vanuatu. The genetic diversity for the Indonesian cultivated (2x/diploid) and wild groups obtained the values of 0.11 and 0.15, respectively, which were the highest values. Their study further revealed that Indonesia holds the highest gene diversities among the taro cultivars and accessions. The SSRs have also been used to analyze diversity in taro found in Northern Queensland, Australia, to demonstrate that natural breeding and population spread occurs in Australian wild taro (Hunt *et al*., 2013). The SSRs were also considered as useful markers to study the genetic diversity in taro in China (Lu *et al*., 2011; You *et al*., 2015).

The use of ISSR techniques in this study on genetic diversity are considered as efficient since it is fast, economical, having high reproductivity for the inter-microsatellites on several locus in genomes. The ISSR analysis requires small amount of DNA and does not require information about previous genomes (Monfared *et al.*, 2018). Moreover, the ISSR markers could improve the weaknesses of RAPD techniques because ISSR is more sensitive to detect genetic diversity at low level, but it is as easy and economical as RAPD technique (Bradford, 2008). Several studies on various plant species have been carried out using the ISSR technique (Poyraz, 2016; Cui *et al*., 2017; Nudin *et al*., 2017; Abdel-Latief and Hewedy, 2018; Harsono *et al*., 2018; Monfared *et al*., 2018; Sanchez-Velazquez and Ramos-Diaz, 2018).

Therefore, based on above considerations, the present study was planned with the aim to investigate the genetic diversity in taro collected several Indonesian provinces using the ISSR technique. The information gathered from such analyses of genetic diversity could be used for further improvement and future conservation of the taro genetic resources.

MATERIALS AND METHODS

Plant material

This study was conducted during July 2017 to June 2018 at the Research Center of Biotechnology, The Indonesian Institute of Sciences (LIPI), Indonesia. Germplasm comprising 50 genotypes (42 cultivars and eight accessions) of taro (*Colocasia esculenta* L. Schott) collected from seven provinces of Indonesia and have been planted in germplasm garden (Table 1).

DNA isolation and quantification

Total genomic DNA was isolated from the leaves by using CTAB protocol (Sharma *et al.*, 2008). The quality of isolated DNA was checked through electrophoresis on 1% agarose gel, 0.5x buffer TBE, 70 volt for 45 minutes and was visualized using *Biodocanalyser.* The DNA quantity and purity were estimated by using nanodrop, and template DNA was adjusted to 100 ng/ul.

ISSR amplification

The DNA template was amplified using PCR Thermal Cycler Biometra, under following condition: One denaturation cycles (94^{o}C, for 3 minutes), followed by 35 amplification cycles (denaturation 94^{o}C for 30 seconds, annealing 30-34^{o}C (adapted to primer) 30 seconds, elongation 72^{o}C for 1 minute, and extended elongation for 10 minutes in 72^{o}C. Total reaction volume (10µl) consisted of 1 µl DNA genomic DNA 100 ng/µL, 1 µl DNA reaction buffer 10x, 0.2 µl dNTPs 10 mM, 0.4 µl ISSR primer 10 µM/ul, 0.04 µl DNA Taq polymerase enzyme (5U/µl), and ddH_2O was added until the total reaction volume was reached. In this study 30 ISSR primers were used (Table 2). The PCR products were then electrophoresed for 120 minutes in 50 volt on 2% of agarose gel in 1x TBE Buffer containing 4% SYBR safe *DNA Stain*. The agarose gel was then visualized in *Syngene G: Box Gel Image Analysis System* machine.

Data analysis

The DNA amplification products (electrophoregram) were analyzed using gel analyzer. The scoring was conducted on bands position of all the accession numbers and primers used were scored by presence (1) and absence (0). Scoring of the data was compiled by using Microsoft Excel in binary matrix form. The data were further analyzed by using SAHN-UPGMA (Sequential Agglomerative Hierarchical and Nested-Unweighted Pair Group Method Arithmetic) on PAST software ver. 3.25 (Hammer *et al*., 2001). The results of the analysis were further presented in dendrogram. The scoring results were also analyzed using *Power Marker 3.25* software to calculate statistic parameters on the number of alleles, main alleles frequency, genetic diversity, heterozygous, and polymorphic information content (PIC) values were determined by the ISSR marker (Liu and Muse, 2005).

RESULTS

ISSR amplification

Results exhibited that 17 out of 30 ISSR primers revealed the amplification products in 50 taro genotypes (42 cultivars and eight

Table 1. Taro cultivars and accessions with their locations of collection in Indonesia.

No.	Cultivar	Origin		No.	Cultivar	Origin	
		City	Province			City	Province
1	T. Bogor	Pandeglang	Banten	26	T. Kutil	Saketi	Banten
2	T. Ketan	Saketi	Banten	27	T. Paris	Bogor	West Java
3	T. Ketan	Bogor	West Java	28	T. Lompong	Bogor	West Java
4	T. Hideung	Lebak	Banten	29	T. Berod	-	-
5	T. Ketan	Saketi	Banten	30	T. Loma	Bogor	West Java
6	T. Burkok	-	-	31	T. Pandan	Bogor	West Java
7	T. Berod	Lebak	Banten	32	T. Kaliurang (Acc.)	Yogyakarta	DI Yogyakarta
8	T. Gunung (Acc.)	Lebak	Banten	33	T. Sutra Bodas	Bogor	West Java
9	T. Ketan Hideung	Bogor	West Java	34	T. Bitik	Tator	South Sulawesi
10	T. Bentul	-	-	35	T. Bolang Hideung	Bogor	West Java
11	T. Bentul	-	-	36	Talas Papua (Acc.)	Merauke	Papua
12	T. Bentul	-	-	37	T. Bentul Hitam	Bogor	West Java
13	T. Lahun Indung	Bandung	West Java	38	T. Bogor	Bogor	West Java
14	T. Jahe	Sumedang	West Java	39	T. Lampung	Bogor	West Java
15	T. Gelo	Lebak	Banten	40	T. Semir	Sumedang	West Java
16	T. Apu	Lebak	Banten	41	T. Pontianak (Acc.)	Pontianak	West Kalimantan
17	T. Kudo	Pontianak	West Kalimantan	42	T. Pak Daud (Acc.)	Mamasa	West Sulawesi
18	T. Andong	Lebak	Banten	43	T. Makki (Acc.)	Mamasa	West Sulawesi
19	T. Burkok	-	-	44	T. Kaluku (Acc.)	Mamasa	West Sulawesi
20	T. Bogor	Bogor	West Java	45	T. LIPI (Acc.)	Yogyakarta	D. I. Yogyakarta
21	T. Bogor	Bogor	West Java	46	T. Bentul	Cibinong	West Java
22	T. Hirik/Birik	Bogor	West Java	47	T. Sutra	-	-
23	T. Sutra	Lebak	Banten	48	T. Ketan Hitam	-	-
24	T. Lompong Paris	Cianjur	West Java	49	T. Bogor	-	-
25	T. Gambir	Sumedang	West Java	50	T. Berod	-	-

Note: T = Taro, Acc. = Accession

accessions). The quality of DNA banding patterns was revealed clearly and could be analyzed further (Table 3 and Figure 1). The total identified alleles were 218 at an average of 12.82 alleles per locus. The ISSR primer 17899A produced three alleles which was the lowest. Whereas, ISSR primers i.e., HB14, 844A and 814 produced 25 alleles, which were the highest alleles number. The highest number of amplified alleles indicated the high diversity in the analyzed taro cultivars and accessions. In this study, the average frequency of main alleles was 48%, ranging from 37% (locus HB 12) to 55% (locus Primer 2). The lowest genetic diversity value was 0.60 (locus 17899A) while the highest value was 0.75 (locus 844B and HB 10), and the average was 0.70. Heterozygosity corresponds to a probability that two alleles taken at random from a population can be distinguished by using the marker (Dangi *et al*., 2004). Heterozygosity values were as many as 0.89 (HB 12) to 1.0 (HB 8, HB 9, HB 10, HB 11, HB 14, HB 15, 814, 17898A, and 17898B) with an average of 0.98. The higher value of heterozygosity indicated the higher genetic diversity.

Polymorphism information content (PIC)

The polymorphism information content (PIC) values indicate high or low level of markers polymorphism. According to Botstein *et al*. (1980), PIC > 0.5 was considered very informative; intermediate information at 0.5 > PIC

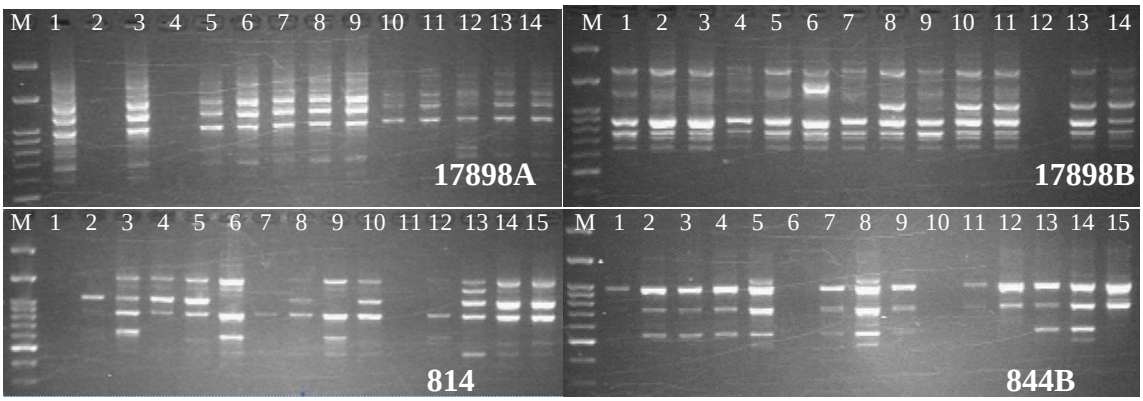

Figure 1. DNA amplification products (electrophoregram) using four ISSR primers. Note: M= marker 100 bp; 1-15 = sample.

Table 2. ISSR primers used to analyze the 50 cultivars and accessions of taro in Indonesia.

No.	Primers name	DNA repeats	Primers sequence (5′ – 3′)
1	UBC-11	(AT8T)	AT AT AT AT AT AT AT AT T
2	UBC-14	(CT8A)	CT CT CT CT CT CT CT CT A
3	UBC-15	(CT8G)	CT CT CT CT CT CT CT CT G
4	UBC-16	(CA8)	CA CA CA CA CA CA CA CA
5	UBC-19	(GT8A)	GT GT GT GT GT GT GT GT A
6	UBC-20	(GT8C)	GT GT GT GT GT GT GT GT C
7	UBC-21	(GT8T)	GT GT GT GT GT GT GT GT T
8	UBC-65	(CCG6)	CCG CCG CCG CCG CCG CCG
9	UBC-66	(CTC6)	CTC CTC CTC CTC CTC CTC
10	UBC-67	(GGC6)	GGC GGC GGC GGC GGC GGC
11	UBC-70	(AT8G)	AT AT AT AT AT AT AT AT G
12	UBC-86	(AT8C)	AT AT AT AT AT AT AT AT C
13	UBC-95	(GT8GA)	GT GT GT GT GT GT GT GT GA
14	814	(CT)8TG	CT CT CT CT CT CT CT CT TG
15	844A	(CT)8AC	CT CT CT CT CT CT CT CT AC
16	844B	(CT)8GC	CT CT CT CT CT CT CT CT GC
17	17898A	(CA)6AC	CA CA CA CA CA CA AC
18	17898B	(CA)6GT	CA CA CA CA CA CA GT
19	17899A	(CA)6AG	CA CA CA CA CA CA AG
20	17899B	(CA)6GG	CA CA CA CA CA CA GG
21	HB8	(GA)6GG	GA GA GA GA GA GA GG
22	HB10	(GA)6CC	GA GA GA GA GA GA CC
23	HB9	(GT)6GG	GT GT GT GT GT GT GG
24	HB11	(GT)6CC	GT GT GT GT GT GT CC
25	HB12	(CAC)3GC	CAC CAC CAC GC
26	HB13	(GAG)3GC	GAG GAG GAG GC
27	HB14	(CTC)3GC	CTC CTC CTC GC
28	HB15	(GTG)3GC	GTG GTG GTG GC
29	Primer 1	$(GA)_9AT$	GA GA GA GA GA GA GA GA GA AT
30	Primer 2	$(GA)_9AC$	GA GA GA GA GA GA GA GA GA AC

Note: 01-13: Singh *et al*. (2012), 14-28: James *et al*. (2012), 29-30: Singh *et al*. (2008).

Table 3. Number of alleles, frequency of main alleles, genetic diversity, heterozygosity, and the level of polymorphism in 50 taro cultivars and accessions using 17 ISSR primers.

Primers	Number of alleles	Frequency of main alleles	Genetic diversity	Heterozygosity	PIC
HB 8	16	0.50	0.72	1.00	0.70
HB 9	10	0.49	0.67	1.00	0.63
HB 10	8	0.41	0.75	1.00	0.72
HB 11	12	0.48	0.69	1.00	0.65
HB 12	7	0.37	0.74	0.89	0.70
HB 13	5	0.48	0.67	0.96	0.62
HB 14	25	0.49	0.74	1.00	0.73
HB 15	10	0.50	0.68	1.00	0.65
844A	25	0.51	0.72	0.98	0.71
844B	8	0.43	0.75	0.98	0.73
814	25	0.49	0.74	1.00	0.73
17898 A	11	0.50	0.69	1.00	0.66
17898 B	13	0.49	0.71	1.00	0.68
17899 A	3	0.50	0.60	1.00	0.52
17899 B	7	0.52	0.64	0.96	0.58
Primer 2	17	0.55	0.67	0.90	0.66
UBC 15	16	0.51	0.71	0.98	0.70
Total	218	8.22	11.89	16.65	11.37
Mean	12.82	0.48	0.70	0.98	0.67

> 0.25 and PIC < 0.25 were less informative. Whereas, Hildebrand *et al*. (1992) findings revealed the criteria that the PIC value (> 0.7) was more informative, PIC 0.44-0.7 had a medium criterion. Results further indicated that the PIC values ranged from 0.52 to 0.73. The highest PIC value (0.73) was obtained from ISSR markers viz., HB 14, 844B, and 814. On the contrary, the lowest PIC value (0.52) was indicated by ISSR marker 17899A. Based on the criteria of Botstein *et al*. (1980), all the used markers were very informative (PIC > 0.5), whereas according to the Hildebrand *et al*. (1992) criteria there were five markers which were very informative namely HB 10, HB 14, 844A, 844B, and 814 by having PIC values of 0.71 to 0.73.

Cluster analysis

Cluster analysis using SAHN-UPGMA enunciated that the grouping displayed the genetic relationship of taro cultivars and accessions in dendrogram generated by PAST software ver. 3.25 (Figure 2). Based on the dendrogram, four clusters were generated from the similarity coefficient of 67% out of 50 tested cultivars and accessions. Both cluster I and II contain one each cultivar i.e., Bentul (46) and Bitik (34), respectively. Cluster III contained 36 cultivars and seven accessions while cluster IV comprising four cultivars (25, 31, 33, and 49) and one accession (41). The clustering data was not highly associated with the distribution of different provinces of

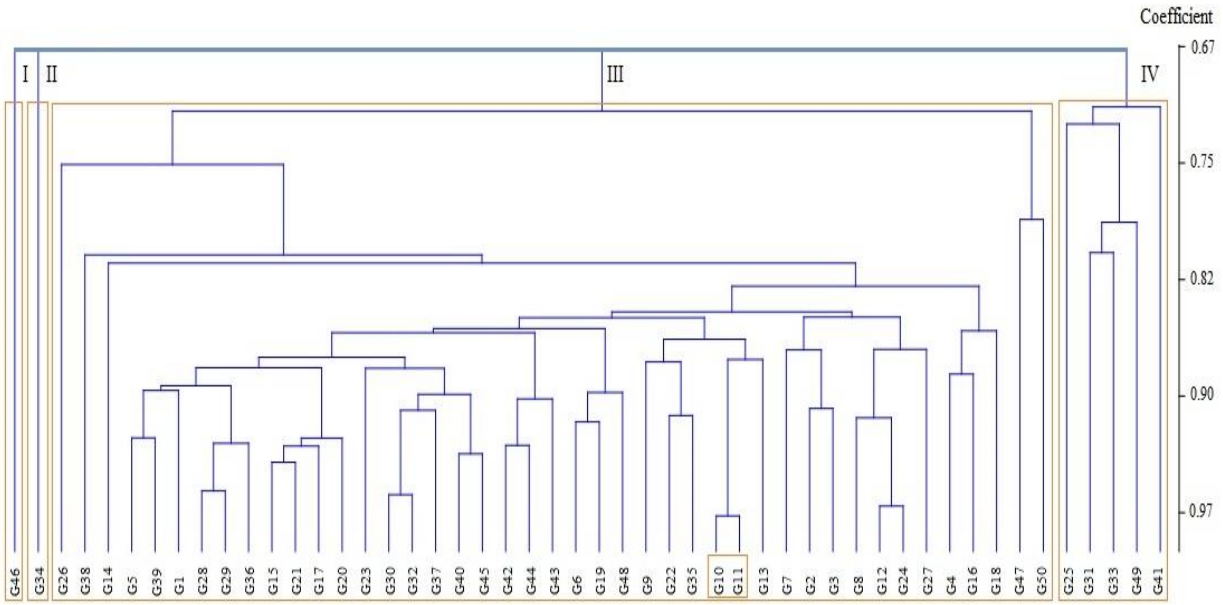

Figure 2. Dendrogram illustrating similarity and cluster grouping generated by UPGMA of 50 Indonesian taro cultivars and accessions based on ISSR markers.
Note: Serial number of a cultivar and accession was same individual plant number as in Table 2.

Indonesia (geographic origin) from taro cultivars. Taro from a province was not only located in one cluster. The taro cultivars and accessions from West Java were spread in clusters I, III and IV. The taro cultivars and accessions from West Kalimantan were also observed in clusters III and IV. Cluster II was from South Sulawesi, Indonesia. The taro cultivars and accessions from Banten, Yogyakarta and Papua, Indonesia were found in cluster III (Table 4).

Location mapping of taro cultivars and accessions

Results revealed that the used 50 taro cultivars and accessions were found to be distributed into seven provinces of Indonesia i.e., West Java, Banten, Yogyakarta, West Kalimantan, West Sulawesi, South Sulawesi, and West Papua (Figure 3). West Java was the leading and had the highest number of taro cultivars and accessions as compared to other six provinces. Furthermore, Bogor City had the highest cultivars and accessions (13) while others locations i.e., Cianjur, Bandung, Toraja, and Merauke contributed one each cultivar/accession. The locations Kaliurang, and Pontianak contributed two each cultivars/accessions. The three other locations i.e., Sumedang, Pandeglang, and Mamasa contributed three each cultivar/accession, and seven cultivars and accessions were found in Lebak province (Table 4). Separation of cultivars and accessions based on clusters, exhibited that some cultivars and accessions were found to be distributed in the same cluster and or in two different clusters (Table 4). For example, Bentul taro cultivars and accessions were found in clusters I and III, Bogor taro cultivars and

Table 4. Cluster analysis of 50 taro cultivars and accessions and their origin based on ISSR markers.

Clusters	Cultivar / Accession numbers	Cultivars and accessions	Origin	
			City	Province
I	46	T. Bentul	Cibinong	West Java
II	34	T. Bitik	Tator	South Sulawesi
III	1	T. Bogor	Pandeglang	Banten
	2	T. Ketan	Saketi	Banten
	3	T. Ketan	Bogor	West Java
	4	T. Hideung	Lebak	Banten
	5	T. Ketan	Saketi	Banten
	6	T. Burkok	-	-
	7	T. Berod	Lebak	Banten
	8	T. Gunung	Lebak	Banten
	9	T. Ketan Hideung	Bogor	West Java
	10	T. Bentul	-	-
	11	T. Bentul	-	-
	12	T. Bentul	-	-
	13	T. Lahun Indung	Bandung	West Java
	14	T. Jahe	Sumedang	West Java
	15	T. Gelo	Lebak	Banten
	16	T. Apu	Lebak	Banten
	17	T. Kudo	Pontianak	West Kalimantan
	18	T. Andong	Lebak	Banten
	19	T. Burkok	-	-
	20	T. Bogor	Bogor	West Java
	21	T. Bogor	Bogor	West Java
	22	T. Hirik/Birik	Bogor	West Java
	23	T. Sutra	Lebak	Banten
	24	T. Lompong Paris	Cianjunr	West Jawa
	26	T. Kutil	Saketi	Banten
	27	T. Paris	Bogor	West Java
	28	T. Lompong	Bogor	West Java
	29	T. Berod	-	-
	30	T. Loma	Bogor	West Java
	32	T. Kaliurang	Yogyakarta	DI Yogyakarta
	35	T. Bolang Hideung	Bogor	West Java
	36	T. Talas Papua	Merauke	Papua
	37	T. Bentul Hitam	Bogor	West Java
	38	T. Bogor	Bogor	West Java
	39	T. Lampung	Bogor	West Java
	40	T. Semir	Sumedang	West Java
	42	T. Pak Daud	Mamasa	West Sulawesi
	43	T. Makki	Mamasa	West Sulawesi
	44	T. Kaluku	Mamasa	West Sulawesi
	45	T. LIPI	Yogyakarta	DI Yogyakarta
	47	T. Sutra	-	-
	48	T. Ketan Hitam	-	-
	50	T. Berod	-	-
IV	25	T.Gambir	Sumedang	West Java
	31	T. Pandan	Bogor	West Java
	33	T. Sutra Bodas	Bogor	West Java
	41	T. Pontianak	Pontianak	West Kalimantan
	49	T. Bogor	-	-

Note: T = Taro

Figure 3. Distribution of seven origin provinces of Indonesia indicated with black circles for collection of taro cultivars and accessions.

accessions were also distributed in two different clusters i.e., clusters III and IV. However, 35 other cultivars and accessions were grouped in the same one cluster.

DISCUSSION

ISSR amplification

The DNA amplification using ISSR primers were applied to analyze the genetic variation and relationship among the 50 taro genotypes (42 cultivars and eight accessions) collected from seven different provinces of Indonesia. Results of PCR revealed that not all the primers used in this study could generate DNA band. As many as 56.7% primers were able to amplify genome DNA of all the tested taro cultivars and accessions. Therefore, the preliminary study of primers used to generate the consistent DNA bands could be carried out repeatedly (Singh *et al*., 2012; Poyraz, 2016; Nudin *et al*., 2017). Generally, more DNA markers can be used, that could represent more plant genome (Karsinah *et al*., 2002). The number of alleles was not only influenced by the number of primers, but also by number of samples and cultivars and accessions, as well as how the primers recognized the sequence of DNA complementary on DNA template. There were total of 218 alleles, by average of 12.82. Every cultivar has different number of alleles ranging from 3 to 25 by using different primers.

PCR recapitulation showed that 17 out of 30 ISSR primers were used to have high level of polymorphism. Generally, the produced numbers of DNA products were around four to ten DNA bands which could be analyzed. The three ISSR primers i.e., HB 14, 844B and 814 produced the highest number of polymorphic bands. The annealing temperature played an important role in generating the amplification products. Based on the optimization results, the best annealing temperature towards

primers used in this study was around 30 to 34°C. Beside of primers, the DNA quality and quantity, the other components in PCR reactions were also important which determined the successful amplifications. The different primers for same sample would produce different electropherogram performances and the opposite. Principally, the primers only amplified the locus which was suitable for sequence bases. Therefore, be able to observe the wide diversity from a population, the number of primers should be enhanced. By using various primers, the opportunity to obtain the amplification products from several loci in genome was higher (Anggraheni and Mulyaningsih, 2018).

Eleven microsatellite markers (SSRs) were used to study the cultivated taro global diversity in 19 countries in Asia, the Pacific, Africa and America (Chair *et al*., 2016). The highest genetic diversity was observed in Asia, especially in India. However, taro has been diversified in Asia and the Pacific mostly via sexual reproduction, and the clonal reproduction with mutation appeared predominant in Africa and America. Two groups of diploids were identified as one from the Asia-Pacific region and the other from India but admixed cultivars and accessions were also found in two genetic pools. In West Africa, most of the taro cultivars and accessions were found to have been introduced from India. As expected, taro cultivars and accessions in Madagascar were found to be originated from India and Indonesia but, surprisingly, the South African taro cultivars and accessions shared lineages with Japan. In the Caribbean Islands, the taro cultivars and accessions were found to be originated from the Pacific, while in Costa Rica the taro cultivars and accessions were found to be from India or Asia.

Diverse taro cultivars and accessions revealed higher genetic diversity. The ISSR markers applied were able to distinguish between homozygote and heterozygote taro cultivars and accessions. The higher heterozygote value means the higher genetic diversity. However, the marker with the PIC value of more than 0.7 demonstrated that it was informative (Hildebrand *et al*., 1992). It could become the candidate of identification marker to distinguish between the taro cultivars and accessions. Singh *et al*. (2013) findings showed that PIC value was influenced by many factors i.e., techniques of plant propagation, genetic diversity in collection, size of the collection, genotyping method, and the location of markers in genome.

Cluster analysis

The closest genetic distance with the similarity of 98% was observed in T. Bentul cultivars (number 10 and 11) which were in cluster III, without knowing the origin. It was suspected that these two Bentul cultivars might be sister lines, although it required some more analysis. Moreover, the lowest similarity (72%) was found in the cluster III between number 26 (Kutil) and 50 (Berod). The highest similarity (81%) in the cluster IV was found in numbers 31 and 33, namely Pandan and Sutra Bodas cultivars. The others clusters i.e., I, and II had the low similarity level (less than 65%) towards all numbers of tested cultivars and accessions. The farthest similarity in cultivar number 46 (Bentul) and/or 34 (Bitik) was towards cultivar number 41 (Pontianak). Results of

cluster analysis based on the DNA amplification were correlated with morphological, agronomic, and physiological observations (Singh *et al.,* 2008; Singh *et al*., 2012; Nudin *et al*., 2017; Fang *et al.,* 2018). Although such type of fidings about genetic diversity were not always seen or apparently correlated with morphological observations, and unfortunately, in this manuscript cannot be explained the results about the phenotypic observations.

Location mapping of taro cultivars and accessions

Taro cultivars and accessions in Asia displayed the greatest genetic diversity. The high diversity and variation may be occurred with crosses, somaclonal variation, and mutation (Asadi, 2013; Helmkampf *et al.,* 2018). Amadi *et al.,* (2015) reported that the seedling from nine taro crosses expected to be genetically different and potentially improved. Past findings revealed that somaclonal variation and mutation also contributed to taro diversity (Vandenbroucke *et al.,* 2016). Mandal *et al.* (2013) reported that somaclonal variation involving in phenotypic traits diversity and somatic mutations can occur regularly and retain that makes taro morphologically different from others.

Based on the existing taro collection, the province West Java has the highest diversity of taro germplasm than other provinces of Indonesia. Furthermore, Bogor, Indonesia has the highest diversity of taro, and therefore, Bogor is well known as the city of taro. Bogor has determined taro as one of the city icon. Bogor agro-ecological conditions with sufficient rainfall, fertile soil, and humid with the temperature around 21-27 ºC, make it suitable for taro to grow and develop better in this area (Andarini and Risliawati, 2018). Some of the commercial cultivar of taro was also found in this city with various names. According to this study, various commercial cultivars names have been known so far and could be proven based on their genetics. In addition to Bogor, the two other provinces i.e., Lebak, and Banten have also some amount of superior taro cultivars and accessions. In this study, the tested cultivars and accessions of taro were collected at the *ex situ* Germplasm Garden, Research Center of Biotechnology, LIPI, Indonesia. However, this collection was less than their existence in the original habitat.

Based on cluster analysis about the relationship between taro cultivars and accessions and the province of origin, it was found that there was only one cultivar (T. Bentul) in cluster I which was from Cibinong, West Java. In cluster II, there was only T. Bitik from Toraja, South Sulawesi. In cluster III, there were cultivars and accessions namely T. Bogor, T. Ketan, T. Ketan, T. Hideung, T. Ketan, T. Burkok, T. Berod, T. Gunung, T. Ketan Hideung, T. Bentul, T. Bentul, T. Bentul, T. Lahun Indung, T. Jahe, T. Gelo, T. Apu, T. Kudo, T. Andong, T. Burkok, T. Bogor, T. Bogor, T. Hirik/Birik, T. Sutra, T. Lompong Paris, T. Kutil, T. Paris, T. Lompong, T. Berod, T. Loma, T. Kaliurang, T. Bolang Hideung, T.S. Papua, T. Bentul Hitam, T Bogor, T. Lampung, T. Semir, T. Pak Daud, T. Makki, T. Kaluku, T. LIPI, Talas Sutra, T. Ketan Hitam, and T. Berod which were originally from various province, namely West Java, Banten, Yogyakarta, West Kalimantan, West Sulawesi, and Papua, except T. Burkok

(6 and 19), T. Bentul (10,11, and 12), T. Berod (29 and 50), Talas Sutra (47), and Ketan Hitam (48) whose original habitat was not identified.

In cluster IV, the cultivars and accessions viz., T. Gambir, T. Bogor, T. Pontianak, T.S. Bodas, and T. Pandan belongs to West Java and West Kalimantan. Based on this phenomenon, it was assumed that ISSR analysis was only influenced by genetic factors. The location difference did not correlate to the genetic clustering. Although the distance between the provinces is much more, even separated by sea, however, the genetic distance of taro cultivars and accessions was close to each other. On contrary, if the ISSR analysis was influenced by environmental factors, the clustering would be based on the region and province that taro coming from.

Moreover, if the cluster analysis was found correlated to cultivars and accessions, it revealed that not all the cultivars and accessions with the same name would be in the same cluster. For example, T. Bentul cultivar was distributed in cluster I (number 46) and III (number 10, 11, and 12). Considering the genetic distance range, the taro genotypes with same names were probably different cultivars and accessions. Especially for number 10, 11, and 12, there were no information about the original province. Taro cultivars and accessions T. Bogor showed unique position in cluster analysis, and distributed in clusters III (1, 20, 21, and 38) and IV (49). This study revealed that molecular markers could be effectively used to explore the genetic variability among the taro cultivars and accessions particularly origin sample grouping. This information could become the reference of taro breeding program to improve certain characters in term of genetic index value, as well as to conserve the diversity in taro germplasm.

CONCLUSION

Results revealed that 17 (56.7%) out of 30 ISSR markers were able to analyze the genetic variability in 50 taro genotypes (42 cultivars and eight accessions) collected from seven provinces of Indonesia. However, eight markers (HB 8, HB 10, HB 12, HB 14, 844A, 844B, 814, and UBC 15) by having PIC score of more than 0.7 could be effectively used to distinguish the taro cultivars and accessions. A total of 218 alleles with an average of 12.82 alleles were identified, and various numbers of alleles were detected for each taro cultivar. The closest genetic distance (98%) was found on Bentul cultivar numbers 10 and 11, and might be the sister lines. On the contrary, the lowest similarity coefficient (65%) was found in cultivar numbers 46 (Bentul) and 34 (Bitik). The province West Java has the highest genetic diversity of taro germplasm than other six provinces. Cluster analysis demonstrated that different provinces did not correlate with genetic clustering. Cluster analysis which was correlated to taro genotypes revealed that not all the cultivars and accessions with same name would be in the same cluster. Therefore, ISSR molecular markers were found effective for genetic analysis of taro cultivars and accessions collected from different provinces of Indonesia.

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