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GENETIC VARIABILITY AND CLASSIFICATION OF INDONESIAN YAMS (*Dioscorea* spp.) BASED ON RAPD ANALYSIS

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SUMMARY

Thirty-two Indonesian yam cultivars comprising 24 cultivars of water yam (*D. alata*), 3 cultivars of *D. esculenta*, and 5 species *Dioscorea* were analyzed for genetic variability by random amplified polymorphic DNA (RAPD) markers. The results showed that water yam cultivars were distinct, compared to the other *Dioscorea* species. Water yam cultivars from Sumatera, Java, Kalimantan and Celebes formed a different group compared to Moluccas, West Papua and Nusa Tenggara that have high genetic similarity. Water yam cultivars from Sumatera, Java, Kalimantan and Celebes consist of 'green' and 'purplish-red' group cultivars. The 'green' group cultivar has 3 subgroups namely: 'white ovate-irregular', 'white-yellow short-long cylindrical' and 'white flesh bottle' tuber. The 'purplish-red' cultivar group has 3 subgroups: 'yellow-purple ovate-cylindrical', 'yellow ovate-oblong' and 'white with purple ring tuber flesh' cultivars. The specific characteristics of *D. alata* 'green' group cultivar are light to dark brown tuber skin, and purple for 'purplish-red' cultivar group. *D. cyllindrica*, *Dioscorea* sp. and *D. hispida* have high similarity on RAPD marker, with cylindrical stem and spine; while *ubiopa'* (Central Celebes) and *gembili* (Java) morphologically including to *D. esculenta*.

Key words: *Dioscorea* spp., RAPD, intraspecies, classification, Indonesia

Key findings: This research is very important basic information about Indonesian yams which has potential to be applied in a commercial yam breeding program.

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INTRODUCTION

Water yam (*Dioscorea alata* L.) is a crop in the family of *Dioscoreaceae* (Monocotyledoneae) (Backer and Bakhuizen van den Brink, 1968; Jud *et al*., 1999). It is an old tuber crop that is native to the Southeast Asian region. From a long cultivation history, water yam became an important cultivated species of yam (*Dioscorea* spp.) in West Africa, Caribbean, Indochina and South Pacific Islands (Onweume and Ganga, 1996; Lebot *et al*., 1998; Hasan *et al*., 2006). Morphological variability studies of water yam demonstrate that the variation was enormous within the species, and that characteristics always overlapped within and between populations leading to confusion in distinguishing genotypes (Hasan *et al*., 2006; Malapa *et al*., 2005).

In Indonesia, water yam was considered to be a minor crop, and was traditionally cultivated in villages, mainly Java, Celebes, and Papua. The cultivated variety (cultivar) of water yam in Indonesia has also been representing a wide range of morphological variation that can provide an important source of genetic material for selection and improvement (Onweume and Ganga, 1996). The tubers form an edible part of plant and vary in shape and flesh color (Onweume and Ganga, 1996; Hasan *et al*., 2006). Tuber shape is either irregular with branch or lobes, single oblong, elongated to cylindrical, ovate or rounded, with white, yellowish white, purplish red, or purple tuber flesh color. In Indonesia, based on tuber colors, the species was simply categorized into white to yellow and purple groups which corresponds to green and purple color of leaves and stem nodes (Shiwachi *et al.*, 2000; Hasan *et al.*, 2006). It is irregular white and purple, oblong white and purple, rounded white and purple, obovate white, yellow, and purple, and cylindrical white, yellow, and purple (Purnomo *et al*., 2012).

Intraspecific classification is important to identify the germplasm, including cultivar of plant, to determine simple group and subgroup (as cultivated taxon), in the open classification compared to the weedy and wild type of cultivated plant in their species population or closed relation species (Hawkes, 1986; Harland and de Wet, 1986).

Presently, with the development of biotechnology techniques such as the molecular marker methods including random amplified polymorphic DNA (RAPD) based on polymerase chain reaction (PCR) have provided many methods for analyzing genetic diversity in plants. The RAPD method has been suggested to be a reliable and effective method with advantages including lower labor costs and less time consuming for genetic diversity analysis in plants (William *et al.,* 1990). The RAPD technique was used to distinguish individuals and cultivars in many crop species including water yam (Asemota *et al.*, 1996; Lebot *et al.*, 1998; Jui-Seng Lay *et al.*, 2005). The recent yam molecular study using RAPD markers could distinguish water yam, Chinese yam (*D. opposita*) and jinen-jo (*D. japonica*) (Shiwachi *et al.*, 2000). RAPD analysis was conducted to detect the genetic variability of water yam cultivars in Malaysia (Hasan *et al.*, 2006). Identification of white/yellow Guinea yam *D. cayenensis/rotunda complex* was conducted based on RAPD markers used local landraces in Africa and the results show that water yam has the largest variation compared to the other species (Dansi *et al.,* 2000; Zannou *et al.,* 2009). In Taiwan, identification of 9 species of yams including water yam was conducted using RAPD markers, and the results showed that water yam has the largest variation (Jui-Seng Lai *et al.*, 2005). Based on indicated references above, research on Indonesian water yam cultivars is still limited.

This study describes an investigation of the genetic variability based on RAPD markers to identify and classify Indonesian water yam cultivars. And the cultivars, and some species of yams in Indonesia were also observed for additional information about genetic variability from water yam.

MATERIALS AND METHODS

Plant material

A total of 24 water yam accessions 01, 05, 06, 10, 17, 21, 25, 29, 35, 37, 42, 48, 50, 52, 55, 62, 64, 70, 138, 139, 140, 141, 142 and 144 were used in this study and 8 accessions also used as 78 (*D. bulbifera*), 83 (*D. pentaphylla*), 96, 102 and 105 (*D. esculenta*), 107 (unkown; *Dioscorea* sp.), 112 (*D. cyllindrica*), 132 (*D. hispida*) representing various localities in Indonesia and used in this study. Yam accessions collection was conducted in 2009 to 2010 during the tuber harvesting season (October - December), and tubers were cultivated in the field as living collections, and some accessions in grown in the bush or domestically cultivated in home gardens were collected directly from some Indonesia Islands. Determination of accessions were used in this study based on the results of genetic variability, to compare the clusters formed morphologically (Purnomo *et al*., 2012). The accession number, local name, origin, and morphological characters of samples are listed in Table 1 and the origin of accessions indicated on Figure 1.

Table 1. Accession number, cultivar (local names), origin of accession, morphological characters of Indonesia *Dioscorea* spp, and *D. alata* cultivars.

A.N.	Local name of cultivar (species)	Origin of accession	Morphological characters (Stem node, stipule, leaf nerve color), (tuber shape), and (tuber color)
1	Uwi beras (*D. alata*)	Central Java, West Indonesia	Green, oblong, white
5	Uwi elus (*D. alata*)	Central Java, West Indonesia	Light green, rounded, white
6	Uwi alas(*D. alata*)	Central Java, West Indonesia	Green, rounded, white
10	Uwi putih(*D. alata*)	Central Java, West Indonesia	Green, rounded, white
17	Ubi putih(*D. alata*)	Central Celebes, East Indonesia	Green, rounded to cyllindrical, white
21	Uwi legi (*D. alata*)	Central Java, West Indonesia	Green, oblong, white
25	*Uwi butun* (*D. alata*)	Central Java, West Indonesia	Green, ob-ovate, yellowish white
29	*Uwi Luyung putih* (*D. alata*)	Central Java, West Indonesia	Green, cylindrical, white
35	Uwi Luyung kuning (*D. alata*)	Central Java, West Indonesia	Green, cylindrical, yellow
37	Uwi ulo(*D. alata*)	Central Java, West Indonesia	Green, cylindrical plat, white to yellow
42	Uwi kuning(*D. alata*)	Central Java, West Indonesia	Green, ob-ovate, yellow
48	Uwi bangkulit (*D. alata*)	South Kalimantan, West Indonesia	Purplish red, ob-ovate, white with purple outer ring
50	Uwi Luyung senggani (*D. alata*)	Central Java, West Indonesia	Purplish red, cylindrical, dark purple
52	Owe senggani (*D. alata*)	Central Java, West Indonesia	Purplish red, ob-ovate, purple
55	Uwi ungu (*D. alata*)	South Kalimantan, West Indonesia	Purplish red, irregular with branches, dark purple
62	Obi item (*D. alata*)	Pamekasan, Madura	Purplish red, oblong, purple with blackish spot
64	Obi violet(*D. alata*)	Bangkalan, Madura	Purplish red, oblong,, purple
70	Ubi ungu(*D. alata*)	Centre Celebes, East Indonesia	Purplish red, rounded to cylindrical, light purple centre
78	*Gembolo* (*D. bulbifera*)	Central Java, West Indonesia	Dark green, irregular with branches, grayish white
83	Tomboreso or huwi buah (*D. pentaphylla*)	Central Java, West Indonesia	Dark green, irregular with many branches, yellowish white
96	*Gembili* (*D.. esculenta*)	Central Java, West Indonesia	Green, cylindrical, yellowish white
102	*Ubi Opa* (*D. esculenta*)	Central Celebes, East Indonesia	Green,, short cylindrical, yellowish white
105	*Gembili* (*D. esculenta*)	Central Java, West Indonesia	Green, cylindrical, yellowish white
107	*Ubi hutan* (unknown)	Central Celebes, East Indonesia	Light green, cylindrical, white to bone white.
112	*Ubi hutan* (*D.cyllindrica*)	Central Celebes, East Indonesia	Reddish green, cylindrical with branches, reddish white
132	*Gadung* (*D. hispida*)	Lampung, Sumatera, West Indonesia	Green, irregular with many branches, yellowish white to yellow.
138	Ubi ungu(*D. alata*)	Nusa Tenggara, East Indonesia	Purplish green, ob-ovate, purple
139	Ubi putih (*D. alata*)	Nusa Tenggara, East Indonesia	Green, ob-ovate, white
140	Ubi putih (*D. alata*)	Moluccas, East Indonesia	Green, cylindrical, white
141	Ubi ungu(*D. alata*)	Moluccas, East Indonesia	Purplish green, ob-ovate, light purple
142	Ubi ungu (*D. alata*)	West Papua, East Indonesia	Purplish green, oblong to cylindrical, purple
144	Ubi putih (*D. alata*)	West Papua, East Indonesia	Green, ob-ovate, white to yellowish white

Note: A.N. = accession number.

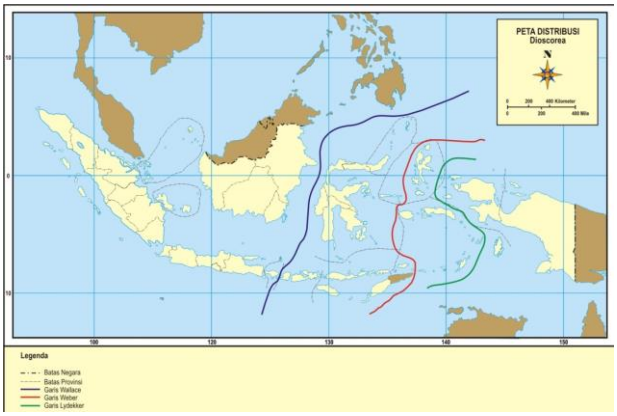

Figure 1. Map of Indonesia showing the origin of the samples (accessions) of *Dioscorea* spp. and *D. alata* cultivars. The black circles show the origin of the samples.

Total leaf DNA isolation

Total DNA was isolated from fresh leaves using Phytopure reagent (Daryono and Natsuaki, 2002). The purity of DNA was determined by the ratio of spectrophotometer reading at 260 nm (optimum absorbance for detecting DNA) and 280 nm (optimum absorbance for detecting protein) using 1.0% (w/v) of DNA samples. The purity of standard DNA reading for 260/280 nm ratios was from 1.8 to 2.2 (Sambrook *et al*., 1989 in Wang *et al*., 2011).

RAPD marker genotyping

Eleven primers such as OPA-01, OPA-02, OPA-10, OPD-03, OPG-02, OPG-03, OPG-05, OPG-06, OPG-08, OPG-13, and OPW-17 (Shiwachi *et al.*, 2000; Taura *et al.*, 2001; Jui-Sheng Lai *et al.*, 2005; Hasan *et al.*, 2006; Zannou *et al*., 2009) were used for polymerase chain reaction (PCR-RAPD) amplification (Table 2).

Table 2. Primers and their nucleotides base sequence used in this study.

No.	Primers	Nucleotide sequence	References
1	OPA-01	5'-CAGGCCCTTC-3'	(Ramser e*t al., 1996;*Hasan *et al*. 2006)
2	OPA-02	5'-TGCCGAGCTG-3'	(Shiwachi *et al*., 2000; Taura *et al*., 2001)
3	OPA-10	5'-GTGATCGCAG-3'	(Jui-Sheng Lai *et al.*, 2005)
4	OPD-03	5'-GTGATCGCAG-3'	(Dansi *et al.*, 2000; Hasan *et al*., 2006;)
5	OPG-02	5'-GGCACTGAGG-3'	(Hasan *et al*., 2006)
6	OPG-03	5'-GAGCCCTCCA-3'	(Hasan *et al*., 2006)
7	OPG-05	5'-CTGAGAGGGA-3'	(Hasan *et al*., 2006)
8	OPG-06	5'-TCACGTCCAC-3'	Dansi *et al.*, 2000; Hasan *et al*., 2006)
9	OPG-08	5'-TCACGTCCAC-3'	(Hasan *et al*., 2006)
10	OPG-13	5'-CTCTCCGCCA-3'	(Hasan *et al*., 2006)
11	OPW-17	5'-GTCCTGGGTT-3'	(Zannou *et al*., 2009)

PCR reactions was composed of 20 µl Mega Mix Blue reagent, 2.5 µl DNA, and 2.5 µl primer and then amplified in the thermo-cycler PCR machine. PCR reaction was conducted at 94^0C for pre-denaturizing 5 minutes, 94^0C denaturizing 1 minute, 36^0C annealing (temperature melting) 3 minutes, 72^0C elongation 2 minutes, 72^0C for post-elongation 10 minute, and it was conducted on 45 cycles. A total of 10 µl of PCR product (RAPD) were separated by electrophoresis on 1.5% agar gel in 1.0 x TBE buffer mixture with 5 µl good view

(modification of ethidium bromide) as a dye, and running at 100 V for 40 minutes. 10 µl DNA ladder was loaded to estimate the sizes of RAPD markers in base pairs. DNA bands were visualized under ultraviolet light and photographed using a digital camera for data recording (Figure 2).

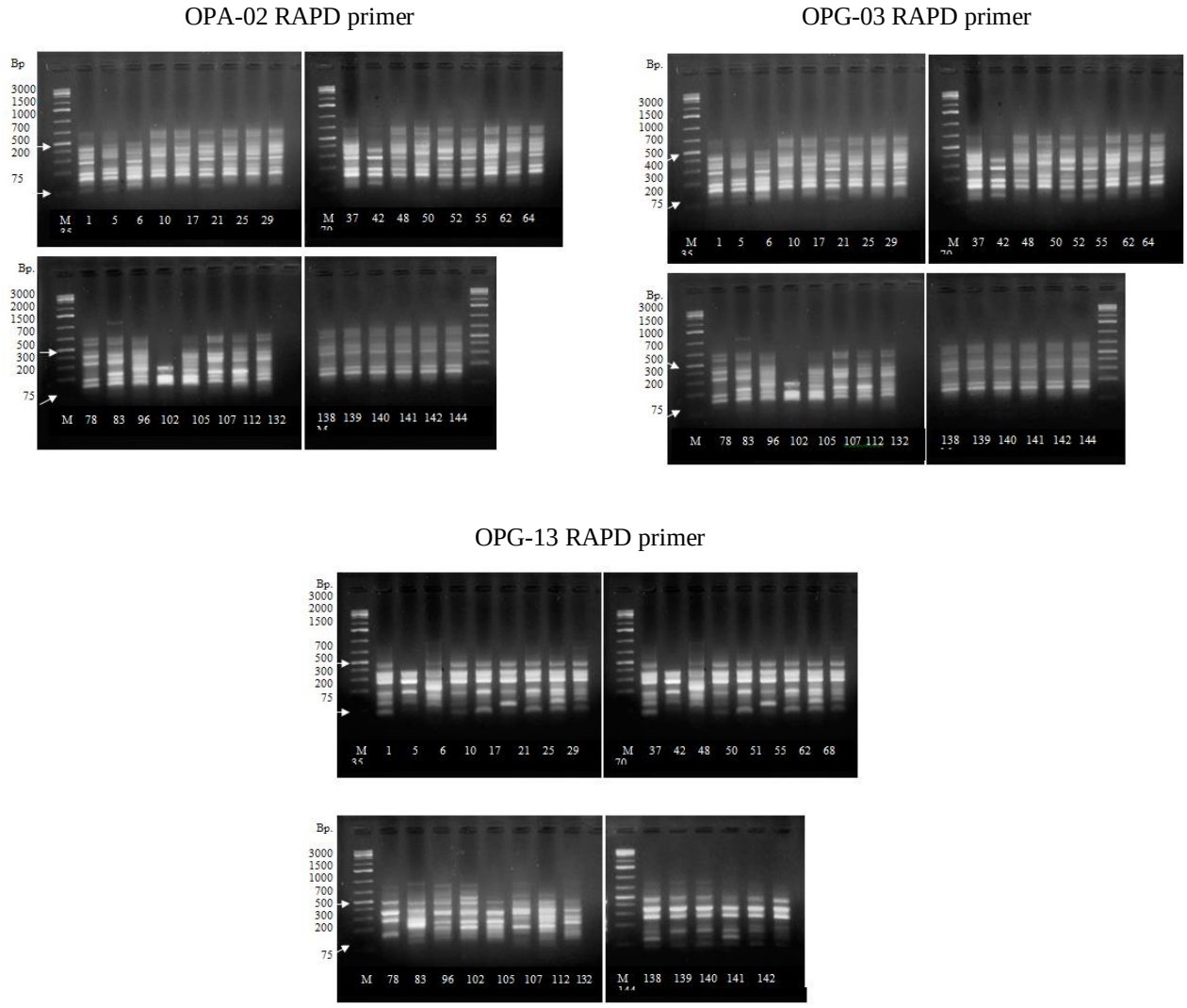

Figure 2. RAPD marker profiles generated by random primer OPA-02, OPG-3, and OPG-13 in 24 Indonesia water yam accessions 01, 05, 06, 10, 17, 21, 25, 29, 35, 37, 42, 48, 50, 52, 55, 62, 64, 70, 138, 139, 140, 141, 142, 144,78 (*D. bulbifera*), 83 (*D. pentaphylla*), 96, 102 and 105 (*D. esculenta*), 107 (unkown; *Dioscorea* sp.), 112 (*D. cyllindrica*), 132 (*D. hispida*).

Data recording and analysis

The position of the RAPD bands in each electrophoresis lane was marked in base pairs (bp), by comparing the marker profile with the DNA ladder. Data recording (scoring) was conducted based on presence (1) or absence (0) of the marker. The binary data matrix of RAPD compiled by the 11 primers of 24 water yam (*D. alata*) cultivars, 3 *D. esculenta* cultivars, and 5 yams species accessions. Similarity coefficients were calculated with Jaccard formula (Sokal and

Sneath, 1962). Based on similarity index data, Unweighted Pair-Group Method Using Arithmetic Average (UPGMA) was used to construct dendrogram using NTSYS.pc. version 2.1 software from Applied Biostatistics Inc., Microsoft (Rohlf, 2000).

RESULTS

Polymorphic fragment of molecular by RAPD analysis

Polymorphic RAPD markers from 11 primers RAPD were listed in Table 3, where primer of OPA-02, OPG-02 and OPG-13 produced the most number of RAPD bands ranging from 75 to 1000 base pairs size.

Table 3. Number of RAPD fragment, fragment size, and number of polymorphic fragment from 11 primers to be used.

No.	Primer	Sequent nucleotide	Number of RAPD fragment	Number of polymorphic RAPD fragment	Number of monomorphic RAPD fragment	Fragment RAPD sized (base pair)
01	OPA-01	5'-CAGGCCCTTC-3'	13	12	1	1000-75
02	OPA-02	5'-TGCCGAGCTG-3'	14	14	0	1000-75
03	OPA-10	5'-GTGATCGCAG-3'	11	11	0	900-100
04	OPD-03	5'-GTGATCGCAG-3'	14	13	1	900-75
05	OPG-02	5'-GGCACTGAGG-3'	16	16	0	1000-75
06	OPG-03	5'-GAGCCCTCCA-3'	12	12	0	900-75
07	OPG-05	5'-CTGAGAGGGA-3'	15	15	0	1500-75
08	OPG-06	5'-TCACGTCCAC-3'	14	13	1	1000-75
09	OPG-08	5'-TCACGTCCAC-3'	13	12	1	900-75
10	OPG-13	5'-CTCTCCGCCA-3'	16	16	0	1000-75
11	OPW-17	5'-GTCCTGGGTT-3'	16	16	0	1500-75

Relationship and classification of Indonesian *D. alata* cultivars and *Dioscorea* spp.

Based on the similarity matrix between accessions (Table 4), with UPGMA cluster analysis method and NTSYSpc2.1 software, 24 water yam accessions and 5 yams species formed a dendrogram (Figure 3). Dendrogram showed that 24 *D. alata* cultivars form the distinct cluster (cluster I), differs from the other species of yams (cluster II.) on 0.58 similarity coefficient (Figure 3). The difference between *D. alata* cultivars and another species were supported by random primer 200 base pair (bp.) of OPA-01, 100 bp. of OPD-03, OPG-06 and OPG-08, 200 bp. of OPG-02 and OPG-06 as deferens fragment RAPD.

The dendrogram also indicates that *D. alata* cultivar that can form 2 clusters based on geographical position were West Indonesia (cluster A) accession from Java, South Kalimantan, Central Celebes (Sulawesi), Lampung (Sumatera) and East Indonesia (cluster B) accession from Papua, Ternate, Nusa Tenggara. Cluster A and B of water yam cultivars have 0.65 coefficient similarity (Figure 3). The difference between A and B clusters were defined by the following RAPD markers: 450 bp - OPA-2; 350 bp - OPA-02; 150 bp - OPA-02; 600 bp - OPG-02; 400bp - OPG-02; 900 bp - OPG-03; 75 bp - OPG-03; 1500 bp - OPG-5; 450 bp - OPG-06, 150 bp - OPG-06; 150 bp - OPG-08, and 300 bp - OPG-13.

The West Indonesian *D. alata* cultivars (cluster A), based on RAPD analysis was divided into 2 groups on 0.75 coefficient similarity (Figure 3). The first (cluster A) morphologically has green stem nodes, upper and lower leaf petiole, leaf nerves, and auricle with white, yellowish- white, to yellow tuber

Table 4. Similarity matrix between accession of *D. alata* cultivars, and *Dioscorea* spp. based on molecular marker using RAPD analysis.

A.N.	1	5	6	10	17	21	25	29	35	37	42	48	50	52	55	62	64	70	78	83	96	102	105	107	112	132	138	139	140	141	142	144
1	100																															
5	88,4	100																														
6	88,4	90,6	100																													
10	78,2	78,9	82,9	100																												
17	85,7	85,1	83,6	85,7	100																											
21	82,9	80,9	79,5	85,7	80,9	100																										
25	88,4	86,3	85	84,3	86,3	87,7	100																									
29	84,3	85,2	83,6	80,2	85,4	85,4	89,1	100																								
35	85,7	85,2	83,6	78,9	85,4	86,3	89,1	94,5	100																							
35	74,1	74,8	70,7	70	74,8	72,1	76,1	77,5	77,5	100																						
42	70	72,1	68,1	59,1	65,3	65,3	66,6	66,6	68,1	78,2	100																					
48	68,1	67,3	65,9	65,3	68,7	68,7	70	70	72,7	73,4	66,6	100																				
50	72,7	73,4	70,7	71,4	76,1	74,8	76,1	76,1	78,9	90,4	76,8	76,1	100																			
52	71,4	69,3	66,6	74,1	74,8	74,8	74,8	76,1	77,5	85,4	75,5	74,8	89,1	100																		
55	71,4	72,1	68	71,4	73,4	74,8	73,4	73,4	73,4	91,8	80,9	74,8	91,8	91,8	100																	
62	72,7	73,4	70,7	74,1	76,1	74,8	77,5	77,5	80,2	85,4	74,1	76,1	90,4	91,8	89,1	100																
64	73,4	71,4	70	73,4	74,1	74,1	76,8	75,5	76,8	85,7	73,4	78,2	89,7	91,1	91,1	93,8	100															
70	72,7	72,1	69,3	70	73,4	72,1	77,5	73,4	76,1	85,4	74,1	77,5	89,1	90,4	90,4	93,1	95,2	100														
78	55,7	53,7	57,8	51,7	55,1	51,1	53,7	52,3	53,7	55,7	54,4	57,8	57,1	54,4	51,7	58,5	57,8	54,4	100													
83	61,9	61,2	63,9	63,2	62,5	65,3	62,5	61,2	62,5	61,9	64,6	68,1	63,2	64,6	63,2	64,6	63,9	63,2	68,1	100												
96	54,4	53,7	56,4	55,7	53,7	55,1	53,7	52,3	53,7	55,7	54,4	64,6	55,7	58,5	55,7	59,8	59,1	58,5	74,1	77,5	100											
102	51,1	53,2	53	44,2	43,5	48,9	48,9	47,6	46,2	55,1	64,6	54,4	52,3	49,6	55,1	49,6	50,3	51,2	58,5	59,1	63,9	100										
105	57,8	61,2	59,8	53,7	55,7	57,1	54,4	55,7	57,1	56,4	61,9	66,6	56,4	57,8	59,1	60,5	61,2	59,1	72,1	76,8	88,4	63,2	100									
107	48,2	50,3	48,9	49,6	50,3	54,4	51,7	48,9	50,3	53,7	53,7	57,1	55,1	57,8	55,1	59,1	59,8	59,1	70,7	71,4	73,4	55,1	75,5	100								
112	51,7	52,3	53,7	51,7	53,7	55,1	52,3	49,6	51,1	58,5	55,7	59,1	57,1	59,8	58,5	61,2	61,9	59,8	75,5	73,4	75,5	55,7	77,5	88,4	100							
132	51,1	54,4	53,2	51,1	53,2	53,2	53,2	50,3	50,3	55,1	56,4	58,5	55,1	56,4	56,4	57,8	58,5	59,1	69,3	72,7	76,1	61,9	78,2	80,9	82,9	100						
138	63,2	61,2	62,5	63,2	66,2	63,9	68,1	63,9	63,9	65,9	60,5	62,5	56,9	67,3	64,6	67,3	68,1	67,3	63,9	65,9	63,9	52,5	63,2	64,6	66,6	65,9	100					
139	59,1	61,2	63,9	64,6	66,6	63,9	65,3	61,2	61,2	65,9	60,5	61,2	65,9	64,6	64,6	65,9	66,6	65,9	68,1	65,9	65,3	52,3	64,6	64,6	68,1	64,6	94,5	100				
140	61,9	62,5	63,9	64,6	66,6	63,9	66,6	62,5	62,5	65,9	63,2	61,2	65,9	65,9	64,6	67,3	68,1	65,9	68,1	67,3	66,6	55,1	64,9	65,9	68,1	65,9	95,9	97,2	100			
141	63,3	62,5	63,9	63,2	66,6	62,5	68,1	63,9	63,9	67,3	63,2	62,5	67,3	68,7	65,9	68,7	70,7	68,7	63,9	67,3	65,3	53,7	65,9	64,6	68,1	70	94,5	90,4	93,1	100		
142	66,1	63,9	66,6	65,9	68,1	65,3	70,7	65,3	65,3	67,3	63,2	62,5	65,9	67,3	64,6	67,3	69,3	67,3	63,9	67,3	62,5	51,2	63,2	61,9	66,6	65,9	93,1	91,8	93,1	95,9	100	
144	66,7	64,6	65,9	65,3	68,7	65,9	72,7	67,3	67,3	66,6	62,5	60,5	65,3	66,6	63,9	66,6	68,7	66,6	63,2	65,3	60,5	48,9	61,2	59,8	64,6	63,9	91,1	89,7	91,1	93,8	97,9	100

A.N. = Accessions number

color with brown tuber skin. It was named ‘green’ cultivar group. The second (cluster B), morphologically have purplish-red stem nodes, upper and lower leaf petiole, leaf nerves, and auricle with white, yellow, violet, purple, purplish- red, and blackish- purple tuber color, with purple tuber skin. It was labeled ‘purplish-red’ cultivar group. The difference between *D. alata* “green” and “purplish-red” cultivar groups, are supported by random primer RAPD fragment on 125 bp of OPD-03, 900 bp of OPG-06, and 300 bp of OPG-06.

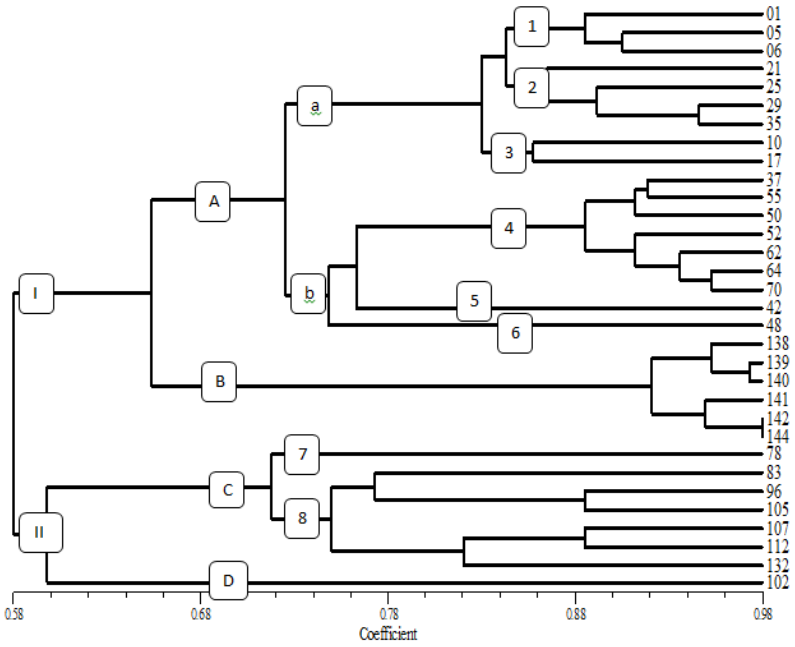

Figure 3. Dendrogram illustrating the variability and similarity relationship of 24 cultivars of Indonesian *D. alata*, 3 cultivar of *D. esculenta*, and 5 yams species. Accessions numbers are listed on the right of the figure (listed in Table 1).

Cluster A, based on RAPD analysis, was divided into 3 subclusters (1-3) on 0.80 - 0.85 coefficient similarity. Subcluster 1 consists of *D. alata* cultivars, with white tuber flesh and single tuber ovate, or with many branches (irregular) tuber shape. This cluster comprises *uwi beras* accession 01 from Bantul, Yogyakarta, *uwi alas* accession 05 from Gunung Kidul, Yogyakarta, *uwi elus* accession 06 from Rembang, nentral Java. It was called *D. alata* 'white ovate-irregular' cultivar group. Subcluster 2 consists of *D. alata* cultivars, with white to yellow tuber flesh and short or long cylindrical tuber shape. This cluster is composed of *uwi legi* accession 21 from Yogyakarta, *uwi butun* 25 from Gunung Kidul, Yogyakarta, *uwi luyung putih* 29 from Bantul, Yogyakarta, *uwi luyung kuning* 35 from Kulon Progo, Yogyakarta on 0.87 coefficient similarity (Figure 3). It was named *D. alata* 'white-yellow short-long cylindrical' cultivar group. Subcluster 3 consists of *D. alata* cultivars, with white tuber flesh and bottle tuber shape, composed of *uwi putih* accession 10 from Purwodadi, Central Java, and *ubi putih* 17 from Buon, Luwuk, Central Celebes on 0.86 coefficient similarity (Figure 3). It was called *D. alata* 'white flesh bottle tuber' cultivar group.

Cluster B, based on RAPD analysis, was divided into 3 subclusters (4-6) on 0.78 coefficient similarity. Subcluster 4 was *D. alata* cultivars with yellow to purple tuber flesh and single tuber ovate to cylindrical tuber shape. This cluster consists of *uwi ulo* accession 37 from Yogyakarta, *uwi ungu* 55 from Demak, Central Java, *uwi luyung senggani* 50 from Bantul, Yogyakarta, *uwi senggani* 52 from Pelaihari, South Kalimantan, *obi item* 62 from Madura, *obi violet* 64 from Madura, and *ubi ungu* 70 from Banggai, Central Celebes (Sulawesi) on 0.86 coefficient similarity (Figure 2). It was called *D. alata* 'yellow-purple ovate-cylindrical' cultivar group. Subcluster 5 was *D. alata* cultivars with yellow tuber flesh and single tuber ovate to oblong tuber shape. This cluster consists of *uwi kuning* accession 42 from Sleman, Yogyakarta only. It was called *D. alata* 'yellow ovate-oblong' cultivar group. Subcluster 6 was *D. alata* cultivars white, with purple ring tuber flesh and single tuber ovate to oblong tuber shape. This cluster consists of *uwi bangkulit*

accession 48 from Batulicin, South Kalimantan. It was called *D. alata* 'white with purple ring tuber' cultivar group.

Cultivar of *uwi ulo* morphologically related to *D. alata* 'green' cultivar group, but based on RAPD analysis including to *D. alata* 'purplish-red' group cultivar. Cultivar *uwi ulo* and *uwi ungu* Demak had the same RAPD fragment on 350 bp. of OPG-13 primer, similar to *D. Alata* 'purplish-red' cultivar group.

Cluster B was the complex of the East Indonesian water yams, consisting of *uwi ungu* accession 138 from Lombok, Nusa Tenggara, *uwi putih* 139 from Lombok, Nusa Tenggara, *uwi putih* 140 from Ternate, Moluccas, *ubi ungu* 141 from Ternate, Moluccas, *ubi ungu*142 from West Papua, *ubi putih*144 from West Papua, and among them have close relationship above 0.92 coefficient similarity (Figure 3).

Intraspecific classification of East Indonesia water yam cultivars can be done morphologically between white and purple cultivar groups (Purnomo *et al*., 2012), but it was not clearly distinguished by RAPD marker. Cultivar of *ubi ungu* accession 138 from Nusa Tenggara on the same cluster with *ubi putih* 139 from Nusa Tenggara, and *ubi putih* 140 from Moluccas Island on 0.96 coefficient similarity (Figure 2). Cultivar of *ubi ungu* accession 141 from Moluccas on same cluster with *ubi ungu* and *ubi putih* 142 and 144 from West Papua on 0.95 coefficient similarity (Figure 3).

Based on RAPD analysis, cluster II was divided into two clusters (C and D). Cluster C consists of *D. bulbifera* accession 78 (subcluster 7), separate from subcluster 8 on 0.72 coefficient similarity. This species morphologically had bulbil in the leaf axils and was separate from the other species. Subcluster 8 consists of *D. pentaphylla* (accession 83), had close relationship to *D. esculenta* (96 and 105) on 0.73 coefficient similarity (Figure 2); the two species morphologically had cylindrical and spiny stem nodes. At subcluster 8, *D. cyllindrica* (accession 112) and *Dioscorea* sp. (107) have close relationship on 0.89 coefficient similarity (Figure 3), morphologically had a difference on the stem shape and color, tuber shape and color. *D. cyllindrica* and *Dioscorea* sp. (unidentified species) formed same group with *D. hispida* (accession 132), which morphologically had strong stem and spines, and on *Dioscorea* sp. had wing stem.

Ubi opak from Central Celebes and *gembili* from Java, Sumatera, and Kalimantan are included in *D. esculenta* species. *Ubi opak* has small tuber size compared to *gembili* with large tuber size, but molecularly by RAPD analysis is on a different group, on 0.60 coefficient similarity (Figure 3). The difference in geographical position can affect tuber size, as an expression of habitat, climate, or molecular structure (Zannou *et al.*, 2009). Muthamia *et al.* (2009) also showed that genetic diversities of yam changed along spatial gradient, most varieties were found in north-east and north-west of Guinea than Central Guinea. There were coastal and terrestrial morphological type water yam in Kenya.

DISCUSSION

Discrimination of water yam (*D. alata*) from other species *Dioscorea* by using RAPD analysis was done successfully. Research on classification of *Dioscorea* spp. in Asia, indicates that *D. alata* can be easily distinguished from *D. opposita* and *D. japonica*. *D. opposita* and *D. japonica* which are the two species that are morphologically difficult to identify (Shiwachi *et al.*, 2000), can be identified by RAPD analysis. Furthermore, *D. alata* can be distinguished from Taiwan and other species using RAPD marker (Jui-Seng Lai *et al.*, 2005). Research in Guinea - Sudan zone of West Africa, indicates that *D. alata* can be clearly identified from *D. cayenensis/rotunda* complex by RAPD analysis (Zannou *et al.*, 2009).

The geographical race (inhabitants) of west and east Indonesian accessions have not supported the distinct *D. alata* morphology. The difference caused by two geographical races can be attributed to climate adaptation, allopatric speciation along Indonesian habitat gradients (Brown and Gibson, 1986). The morphological difference between west and east Indonesia accessions were not clear, but based on RAPD analysis can be identified clearly. The difference in RAPD markers can be caused by chromosome or gene mutation. Allopatric speciation can be identified on gene level that affects plant characteristics leading to loss of natural

hybridization (Swadja & Butlin, 2006). Furthermore, parallel divergence can also be adapted to environmental factors (Butlin *et al*., 2008). Speciation has close connection with population gene flow, and allopatric speciation causes loss of gene flow (Wen Li *et al*., 2010). Research based on RAPD analysis suggests that genetic variability in species of *Dioscorea* follow the spatial gradient along Northeast to Southwest Guinea-Sudan, Benin, West Africa, connect to geographical structure gradient on the research side (Dansi *et al.,* 2000; Zannou *et al.,* 2009). The landrace of water yam could easily be identified by people in the wild (Tamiru *et al*., 2011). By passing the ecotone speciation based on ecologic differentiation following the difference of genetic substances (Thorpe *et al*., 2010).

Morphologically the difference of *D. alata* 'green' and 'purplish-red' cultivar group was supported by RAPD analysis. Research *D. alata* cultivar in Malaysia also classified into the 'white cultivar group' and 'purple cultivar group' that morphologically had various tuber shapes (Hasan *et al*., 2006). The color of stem node, leaf base, tuber, tuber shape, and the appearance of anthocyanin on leaf axils or petiole are importance characteristics to identify the strains of water yam (Shiwachi *et al.,* 2000).

The intraspecific classification of *D. alata* 'green' cultivar group has already been done in Taiwan by RAPD analysis (Jui-Seng Lay *et al*., 2005). Cultivars of *uwi beras, alas, and elus* Indonesia were identical to the 'clumpy yam cultivar' cultivar group. *Uwi luyung putih*, and *luyung kuning* cultivars from Indonesia are identical to 'a long tuber length yam' cultivar group, and *uwi putih* (Purwodadi, Java), and *ubi putih* (Banggai, Central Celebes) cultivars identical to 'a bottle-shape yam cultivar' according to Jui-Sheng Lai *et al*. (2005). Intraspecific classification also was done in Malaysia based on RAPD markers, and cultivars of *uwi beras, alas,* and *elus* Indonesia were identical to 'irregular white' cultivar group, according to Hasan *et al*. (2006).

The intraspecific classification of *D. alata* 'purplish-red'cultivar group was already done in Taiwan by RAPD analysis. Based on this research, *D. alata* cultivars of *uwi ungu, luyung senggani, obi item* (Madura), *obi violet* (Madura) and *ubi ungu* (Banggai) Indonesia were identical to 'a red flesh yam cultivar'cultivar group, *ubi ungu* (Banggai) Indonesia is identical to 'a bottle-shape tuber yam', and also *uwi bangkulit* is identical to 'a white flesh with purple ring and skin yam,' according to Jui-Sheng Lai *et al*. (2005). Intraspecific classification was also done in Malaysia based on RAPD marker, and all the Indonesian cultivars above are identical to *D. alata* 'irregular to oblong purple group' cultivar group, according to Hasan *et al*. (2006).

Based on dendrogram (Figure 3) in cluster I, it is likely that *D. alata* cultivars were selected from the existing variant through clonal propagation. According to the research of van den Brouche *et al*. (2015) and Chair *et al.* (2016), which states that *D. alata* cultivars in Vanuatu is somaclonal engineered (rekayasa) selected as cultivars. Malapa *et al.* (2005) classifies *D. alata* cultivars from various regions including from Vanuatu to 3 major genotypes groups from different geographical areas.

The genetic variability in *D. alata* dominantly is caused by natural gene flow than vegetative reproduction, and by natural hybrid between species of *Dioscorea*. Water yam may be domesticated in Indochina region with *D. hamiltonii* J. D. Hook and *D. persimilis* Prain & Burk. as parents. The cultivation of water yams was started using wild cultigens (Lebot *et al*., 1998), and then continued using vegetative reproduction or clone from tubers.

West and east Indonesian water yam shows different clusters and seem to be following Wallace and Weber line theory. In this research, accession from Celebes belongs to West Indonesia water yam, whereas accession from NusaTenggara belongs to East Indonesia water yam (Susan, 1997; Lee *et al*., 2001). Based on RAPD marker and cluster analysis, Indonesian *D. alata* cultivars can be classified as shown in Table 5.

Water yam in Indonesia belongs to the minor crops category, but the tuber is still used as alternative food, especially in dry areas as carbohydrate substitute in many traditional villages. People commonly take pleasure in water yam with purple tuber color and white sweet tasty cultivar (*uwi legi*). Cultivar *uwi legi* is the best one for Yogyakarta people, because

of the sweet taste when boiled after ripening. Water yam is cultivated as a main crop and is a main food in Banggai culture in District of Banggai Island, Centre of Celebes (Purnomo, 2010). In South Kalimantan and South Sumatera, people do not commonly consume water yams. It was used during traditional ceremonies for opening new plantations (including rubber and oil palm plantation), as one of sources of sacrifice (Purnomo *et al.*, 2012). In recent times, people hardly consume water yam tuber because there are many better crops available, so rice became the main food in Indonesia.

There is a lot of information about the modern uses of water yam starch of water yam that can be made as various food products with nutritious content comparable to sweet potato (Bressnan *et al.*, 2007). Tubers have glucose, maltose, sorbitol content (Balakhrisan *et al.*, 2007), and also alcaloid and steroidal sapogenin that can be used as anti-inflammation and oral contraceptive agents (Judd *et al.*, 1999; Olayemi and Ajaiyeoba, 2007). Tuber flesh has high fiber, carbohydrate, and essential minerals (Wanasundera and Ravindran, 1994; Narina *et al*., 2011). Based on all information, the diversification of food from yam tuber is needed for many purposes. This study provides basic data for water yam cultivar selection and breeding program in Indonesia to produce cultivated seed water yams, which are widely accepted by people.

Table 5. Intraspecific classification (non-formal), group cultivar, sub-group cultivar, local names, and accessions origin of 24 Indonesia water yam cultivars using RAPD marker.

Geographical origin of accessions	Group Cultivars	Sub-group cultivar	Local name of cultivars and collection Sites
West Indonesia	'Green Group'	'white ovate-irregular'	*Uwi beras*, Yogyakarta
			Uwi elus, Rembang Central Java
			Uwi alas, Wonosadi, GunungKidl
		'white-yellow short-long cylindrical'	*Uwi legi*Bantul, Yogyakarta
			Uwi butun, Sleman Yogyakarta
			Uwi luyung putih, Yogyakarta
			Uwi luyung kuning, Yogyakarta
		'white flesh bottle tuber'	*Uwi putih Purwodadi*, C. Java
			Uwi putih, Banggai, C. Sulawesi
	'Purplish-red Group'	'yellow-purple ovate-cylindrical'	*Uwi ulo* Yogyakarta
			Uwi ungu, Demak C. Java
			Uwi luyung Senggani, Yogyakarta
			Uwi senggani, Cianjur, West Java
			Uwi item, Madura
			Uwi violet, Madura
			Uwi ungu, Banggai, C. Sulawesi
		'yellow ovate-oblong'	*Uwi kuning* Lampung, Sumatera
		'white with purple ring'	*Uwi bangkulit*, Yogyakarta
East Indonesia	-	-	*Uwi ungu* Lombok, Nusa Tenggara
			Uwi putih Lombok, Nusa Tenggara
			Ubi putih Ternate, Ambon, Moluccas
			Ubi ungu Ternate, Ambon, Moluccas
			Ubi ungu West Papua
			Ubi putih West Papua

CONCLUSION

Based on RAPD markers, *D. alata* was different from another species *Dioscorea*, Indonesia *D. alata* (water yam) had the largest genetic variability than other species. Indonesian *D. alata* cultivars are classified into 'green' and 'purplish-red' cultivar groups. *D. alata* 'green' cultivar group has 3 cultivar subgroups such as (1) 'white flesh ovate-irregular', (2) 'white-yellow ovate-cylindrical', and (3) 'white flesh bottle tuber'. The 'purplish-red' cultivar group has 3 cultivar subgroups which are (1) 'yellow-purple ovate-cylindrical', (2) 'yellow flesh ovate-oblong', and (3) 'white with purple ring tuber flesh' water yam.

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