J. Agron. Indonesia 40 (1) : 62 - 68 (2012)

Genetic variations of *Amorphophallus variabilis* Blume (Araceae) in Java using AFLP

Edi Santosa[1*], Nobuo Sugiyama[2], Saneyuki Kawabata[3], and Shoko Hikosaka[4]

[1]Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University
Jl. Meranti, Kampus IPB Darmaga 16680, Indonesia
[2]Faculty of Agriculture, Tokyo University of Agriculture, Funako, Atsugi, Kanagawa 243-0034, Japan
[3]Graduate School of Agricultural and Life Sciences, The University of Tokyo
Yayoi 1-1-1 Bunkyoku, Tokyo 113-8657, Japan
[4]Faculty of Agriculture, Chiba University, Chiba, Japan

Received 12 April 2011/Accepted 23 November 2011

ABSTRACT

Amorphophallus variabilis Blume, a member of Araceae, is a native tuber crop in Java, Madura and Kangean Islands, Indonesia. The plant showed high variations in morphology. However, genetic variations at molecular level have not been well studied. Amplified fragment length polymorphism (AFLP) was carried out using 8 primers combination of EcoRI and MseI on 78 accessions collected from 28 sites in Java, Indonesia. Results showed that AFLP markers able to generate polymorphism among accessions. A total of 220 polymorphisms were found. The differences among accessions at the genetic level were high, and 5 clusters were constructed. Grouping was independent of geographical origin, similar to clustering of morphological characteristic of flowers as in the previous report. Accessions from one site composed of one to four different cluster groups, showed that variation in single site was observed. Regarding conservation program of the A. variabilis in natural population, it is reasonable to protect one bigger site rather than many small sites, but it should be recommended to maintain conservation areas in several districts. Further study on population structure should be carried out to explain such variability.

Keywords: genetic diversity, iles-iles, molecular marker, morphology, tuber crop

ABSTRAK

Amorphophallus variabilis, anggota famili Araceae, merupakan tanaman umbi asli di Pulau Jawa, Madura dan kepulauan Kangean, Indonesia. Tanaman ini menunjukkan adanya variasi morfologi yang tinggi, namun variasi genetik menggunakan penanda molekuler masih belum banyak dipelajari. Analisis menggunakan penanda molekuler amplified fragment length polymorphism (AFLP) dilakukan menggunakan 8 pasang primer kombinasi EcoRI dan MseI pada 78 aksesi yang dikumpulkan dari 28 tempat di Jawa, Indonesia. Hasil menujukkan adanya polimorfisme pada aksesi. Total terdapat 220 pita polimorfisme dari seluruh aksesi. Perbedaan genetik antar aksesi tinggi, dan dihasilkan 5 kelompok. Pengelompokan tidak mengikuti pengelompokan berdasarkan asal aksesi, sejalan dengan hasil penelitian sebelumnya menggunakan penanda morfologi. Aksesi dari satu lokasi mengelompok ke dalam satu hingga empat kelompok berbeda, menujukkan adanya variasi genetik dari satu lokasi. Oleh karena itu program konservasi A. variabilis adalah lebih diarahkan untuk melindungi satu lokasi dengan areal yang luas pada kabupaten yang berbeda. Perlu penelitian lebih lanjut struktur populasi tanaman untuk menerangkan faktor yang mempengaruhi keragaman genetik.

Kata kunci: Iles-iles, keragamaan genetik, mofologi, penanda molekuler, umbi-umbian

INTRODUCTION

Amorphophallus variabilis Blume (2n=26) belongs to *Araceae* is native to Java, Madura and Kangean Islands, Indonesia and occurs wild up to 700 to 900 m above sea level (Ohtsuki, 1968; Jansen *et al*., 1996; Yuzammi, 2000). *A. variabilis* is locally called 'iles-iles putih', and *cocooan oray* in Sundanese (Sugiyama and Santosa, 2008). Local people often confused with term of *iles-iles* (*kuning*) for *A. muelleri* Blume and *suweg* for *A. paeoniifolius* Dennst. Nicolson (Sugiyama and Santosa, 2008). Morphologically, *A. variabilis* has no aerial bulbils on the rachis unlike *A. muelleri* (Jansen *et al*., 1996).

A. variabilis has been reported as traditional food in Java, because it corm contains glucomannan about 50% of fresh mass, similar to *A. muelleri* (Sugiyama and Santosa, 2008). It has been reported that corm of *A. variabilis* is suitable as a raw material in food industries. After rice became popular in Indonesia since 1960s, importance of

* Corresponding author. e-mail: editodai@yahoo.com

tuber crops including *Amorphophallus* species has decreased markedly. *A. variabilis*, contained oxalic acid that associated with the acridity and Santosa *et al*. (2004) reported that farmers removed the acridity by drying the corm.

Some characteristics of *A. variabilis* are it has cross pollination, during anthesis, flower produces odor that attracts many Nitidulidae insects, it is a shade loving plant (Sugiyama and Santosa, 2008), and mainly found at edge of teak forests, bamboo trees, coffee plantations, cemeteries, and along river banks thus potential for agroforestry. Furthermore *A. variabilis*, similar to many other *Amorphophallus* species, exhibits dormancy during dry season at a time for harvesting (Santosa *et al*., 2004).

Many authors had reported that *A. variabilis* varies in color of petiole, spot and spadix (Jansen *et al*., 1996; Hetterschied and Ittenbatch, 1996; Yuzammi, 2000). Santosa *et al*. (2004) reported that based on floral and morphological characters, there are four groups and 7 sub groups of *A. variabilis* accessions in West Java.

Several molecular markers have been used in *Araceae* members to resolve complex morphology and variability such as isoenzyme in elephant foot yams (Widjaja and Lester, 1987), microsatellite in *Arisaema* (Nishizawa *et al*., 2003), RAPD in cocoyam (Schnell *et al*., 1999), and *FLORICAULA/LEAFY* (Grob *et al*., 2004).

We used AFLP marker to resolve variability in *A. variabilis* because it generated well markers (Vos *et al*., 1995) for several *Araceae* members such as in *Caladium*, *Alocasia* and *Xanthosoma* (Loh *et al*., 1999; 2000). Moreover, AFLP has been used for determining relationship among tropical plants such as oil palm (Purba *et al*., 2000) and coffee (Steiger, 2002), and among starchy crops such as cassava (Roa *et al*., 1997), radish (Huh and Ohnishi, 2002) and *Dioscorea* (Mignouna *et al*., 2002). The objective of the research was to determine genetic diversity of *A. variabilis* in Java, Indonesia using AFLP.

MATERIALS AND METHODS

Seventy-eight wild accessions from 28 sites of eight districts in West and Central Java were evaluated (Figure 1; Table 1). Plants with corms were collected from July 1998 to December 2002 from a plot of 200 m x 200 m in one site (c.a. 4 ha), with minimum distance between two sites was 1,000 m or after natural border such as a big river. The sites were determined through field tracing of information from farmers and Yuzammi (2000). In each site, 1 to 9 plants were collected depended on its abundance; where each plant was separated by about 100 m apart. Accessions were selected based on characteristic described by Hetterscheid and Ittenbatch (1996) and Jansen *et al*. (1996). Then, the accessions were grown under 50% reduced light intensities in Cikabayan Experimental Farm (6°36'S, 106°48'E, 240 m above sea level), Bogor Agricultural University, Bogor, Indonesia. Identification followed Hetterscheid and Ittenbatch (1996) was conducted in December 2002 to July 2007.

For DNA extraction, young leaflets were taken from plant with petiole diameter at 3 cm from soil surface was minimum 0.8 cm (considered as more than 1 years-old). Petiole diameter was measured at 3 cm from soil surface. Leaflets were cleaned with water, air-dried, removed the main leaflets vein and kept in the plastic bag with silica gel. About a 20 g dry-silica per g fresh sample was used.

DNA was extracted from 0.02 g dry leaves using Nucleon Phytopure extraction kit (Amersam Life Bioscience, USA) with 5% mercaptoethanol, and washed using 70% cool ethanol. During extraction, sticky substance (a kind of polysacharide) was reduced by adding resin (Amersham Bioscience, USA) followed by CI solution (chloroform: IAA, 24:1) and centrifuged. The procedures were repeated until no sticky substances observed. Fishing DNA was carried out if repeated procedures with resin did not satisfy. DNA was diluted with 100 µL water and kept at -20 °C for long storage. The purity of DNA based on A_{260}/A_{280} ratio was 1.77±0.16 (mean±SD) and yielded 12.5±7.3 µg DNA g^{-1} dry weight.

AFLP (Vos *et al.*, 1995) was performed using fluorescent labeled *Eco*RI primer. About 200-500 ng of DNA were digested overnight at 37 °C with 5 U each of *Eco*RI and *Mse*I restriction enzymes. Pre-amplification program consisted of 20 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 120 s, followed by incubation at 60 °C for 30 min. One nucleotide extension of *Eco*-A and *Mse*-C primers was used at pre-amplification.

Primer screening was conducted for 16 primer pairs. Eight primer pairs, i.e., EcoACC-MseCAT, EcoACC-MseCAG, EcoACC-MseCAC, EcoACC-MseCAA, EcoACC-MseCTT, EcoACC-MseCTA, EcoACC-MseCTC and EcoACC-MseCTG were selected, because they yielded many polymorphic bands (Table 2). Amplification was programmed: 1) 10 touchdown cycles of 94 °C for 30 s, annealing temperature which was lowered by 1 °C from 65 °C for each cycle for 30 s, and 72 °C for 120 s; 2) 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 180 s; and 3) one cycle of 60 °C for 30 min, and ended by 4 °C. To each PCR product, a drop of stop solution (98% formamide, 1 mM EDTA, 10 mM Tris-HCl, and small amount bromophenol

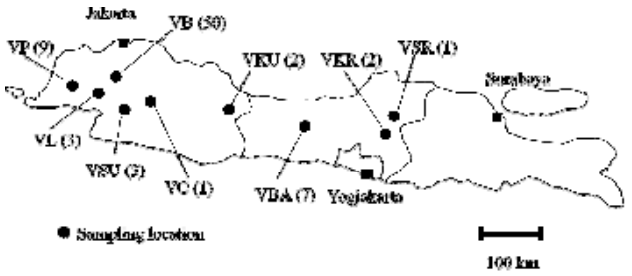

Figure 1. District of origin of *A. variabilis* accessions from Java (l). Value in the parenthesis area number of sites. VB-originated to Bogor, VP-Pandeglang, VL-Lebak, VSU-Sukabumi, VC-Cianjur, VKU-Kuningan, VBA-Banjarnegara, VKR-Karanganyar, VSR-Sragen. ■ = represent cities

blue) (1:1) was added, and the samples were denatured at 95 °C for 3 min prior to electrophoresis. Sample of 2 µL was loaded onto 5% polyacrylamide gel and run with 1x TBE buffer for 24 h at 40 °C. Fragments were separated with sequencer DSQ-2000L (Shimadzu, Japan).

Table 1. Code of accession, site origin and grouping based on AFLP data

Code	Site	Sub-district	Cluster	Code	Site	Sub-district	Cluster
VSU-111	1	Cibadak	1	VB-511	14	Leuwiliang	1
VSU-112	1	Cibadak	3	VB-512	14	Leuwiliang	1
VSU-113	1	Cibadak	3	VB-513	14	Leuwiliang	1
VC-111	2	Selajambe	2	VB-514	14	Leuwiliang	1
VKU-111	3	Ketawang	3	VB-515	14	Leuwiliang	3
VKU-211	4	Darma	2	VB-521	15	Leuwiliang	1
VB-111	5	Citeureup	1	VB-522	15	Leuwiliang	1
VB-112	5	Citeureup	1	VB-611	16	Darmaga	1
VB-121	6	Citeureup	1	VB-612	16	Darmaga	2
VB-122	6	Citeureup	5	VB-613	16	Darmaga	1
VB-211	7	Ciawi	2	VB-614	16	Darmaga	1
VB-212	7	Ciawi	1	VB-615	16	Darmaga	1
VB-213	7	Ciawi	1	VB-621	17	Darmaga	2
VB-311	8	Ciampea	1	VB-711	18	Bogor Tengah	1
VB-312	8	Ciampea	1	VB-712	18	Bogor Tengah	1
VB-313	8	Ciampea	1	VB-811	19	Bogor Timur	2
VB-314	8	Ciampea	1	VB-812	19	Bogor Timur	1
VB-315	8	Ciampea	1	VKR-111	20	Karanganyar	1
VB-321	9	Ciampea	1	VKR-112	20	Karanganyar	3
VB-322	9	Ciampea	3	VSR-111	21	Sragen	1
VB-331	10	Ciampea	1	VBA-111	22	Wanadadi	2
VB-332	10	Ciampea	1	VBA-112	22	Wanadadi	1
VB-333	10	Ciampea	1	VBA-113	22	Wanadadi	3
VB-334	10	Ciampea	3	VBA-311	23	Karangkobar	4
VB-335	10	Ciampea	3	VBA-611	24	Karangkobar	1
VB-341	11	Ciampea	3	VBA-321	25	Banjarmangu	1
VB-342	11	Ciampea	1	VBA-511	26	Klampok	2
VB-343	11	Ciampea	3	VL-111	27	Kertajaya	1
VB-344	11	Ciampea	4	VL-112	27	Kertajaya	1
VB-345	11	Ciampea	1	VL-113	27	Kertajaya	1
VB-351	12	Ciampea	2	VP-111	28	Bantarjaya	1
VB-352	12	Ciampea	1	VP-112	28	Bantarjaya	1
VB-353	12	Ciampea	1	VP-113	28	Bantarjaya	4
VB-354	12	Ciampea	1	VP-114	28	Bantarjaya	3
VB-411	13	Leuwiliang	1	VP-115	28	Bantarjaya	2
VB-412	13	Leuwiliang	1	VP-116	28	Bantarjaya	1
VB-413	13	Leuwiliang	1	VP-117	28	Bantarjaya	1
VB-414	13	Leuwiliang	2	VP-118	28	Bantarjaya	1
VB-415	13	Leuwiliang	2	VP-119	28	Bantarjaya	1

Note: West Java Province: VSU-Sukabumi district, VC-Cianjur district, VKU-Kuningan district and VB-Bogor district; Central Java Province: VKR-Karanganyar district, VSR-Sragen district, and VBA-Banjarnegara district; Banten province: VL-Lebak district and VP-Pandeglang district

Polymorphic bands of AFLP markers were judged manually and scored from clear bands as binary data with presence as "1" and absence as "0". The polymorphic bands shared at more than 75% of total accessions analyzed were used for computing Jaccard's similarity coefficient. Genetic diversity estimates (GDEs) were calculated as 1 - Jaccard's similarity coefficient. Cluster analysis was performed on the similarity matrix employing the UPGMA and presented as dendogram in NTSYSpc ver. 2.20d (Rohlf, 2005). Data of molecular markers were conformed to morphological data of Santosa *et al*. (2004).

RESULTS AND DISCUSSION

AFLP Profiles

Combination of EcoACC with MseCAT, MseCTC, MseCTG, MseCAC and MseCAG produced 35 to 39 bands with 89 to 100% polymorphic (Table 2). Combination of EcoACC with MseCTA, MseCTT and MseCAA produced 31, 27 and 28 bands with more than 92% polymorphic. Totals of 220 polymorphic bands were generated from eight primer combinations.

Shared polymorphic bands ranged from 8% to 89% among accessions. Minimum sharing was 7.9 to 23.6% while maximum sharing was 78.6 to 88.8% (Table 2). Combination of EcoACC x MseCTC and EcoACC x MseCAC primers generated many bands and they were shared in more than 87% accessions.

Cluster Analysis

The accessions could be divided into 5 groups (Figure 2). Group I consisted of 50 accessions followed by Group II and III with 12 accessions in each, and Group IV and V with 3 and 1 accessions, respectively (Table 3). There were some accessions from different sites or districts grouped into one cluster. It was likely that the grouping of most accessions was not associated with geographical origin (sub-district and district) (Table 1). Accessions belonging to Group I mostly came from Bogor. Accessions belonging to Group II and Group III were also dominated by those from Bogor. The three Group IV members were accessions from Bogor, Pandeglang and Banjarnegara. Only one member belonging to Group V (VB-122 from Bogor).

Accessions VB-311, VB-321, VB 411, VB-511, VB-512, VB-513, VB-611 and VB-615 from Bogor were genetically similar to VP-112 and VP-119 from Pandeglang. They also genetically similar to VSU-111 from Sukabumi, and VBA-112 from Banjarnegara districts (Figure 2). In this study, also identified the presence of accessions from different sites but genetically similar (VB-211 similar to VB-621, VKU-111 similar to VSU-112 and VP-114, and VP-116 similar to VB-812). It is probable that, clonal propagation might common in past time.

During field assessment, except for member of Group 1, most accessions were collected from home garden or near resettlement areas. It is probably that those accessions were escape plants. Accessions belonging to Group I were commonly found in most of the collection sites.

In general, accessions collected from one site were mostly grouped into two groups (Table 1). On the other hand, those from sites 11, 22, and 28 were grouped into three to four groups. Samples from Bantarjaya was collected at of bamboo forest, represented natural population. In this sub-district, collected samples of *A. variabilis* belonged into more groups than those of other sites. Huh and Ohnishi (2002) stated that genetic diversity of natural population was high.

Genetic Diversity

The genetic similarity among 78 accessions ranged from 33.8 to 81.6%. Less than 45% of accessions showed similarity of more than 70%. Steiger *et al*. (2002) stated that similarity were considered as low if the similarity was less than 89% among cultivars and less than 50% among species, respectively. Accessions from Banjarnegara, Bogor and Pandeglang were considered more diverse than those from other areas since the similarity value among accessions was low (< 25%).

Table 2. Percentage of polymorphic bands, total bands and percentage of share bands of each Mse primer combination of EcoACC of *A. variabilis* accessions

Mse Primer	Bands		Share bands among accessions (%)		
	Polymorphic (%)	Total	Maximum	Minimum	Average
CTA	100	31	78.65	7.87	31.10
CAT	100	37	78.65	16.85	42.61
CTC	89	35	87.64	23.60	49.57
CTG	92	36	78.65	8.99	43.38
CTT	93	27	82.02	12.36	46.28
CAA	96	28	83.15	23.60	52.89
CAC	95	39	88.76	20.22	46.50
CAG	97	36	85.39	16.85	44.04

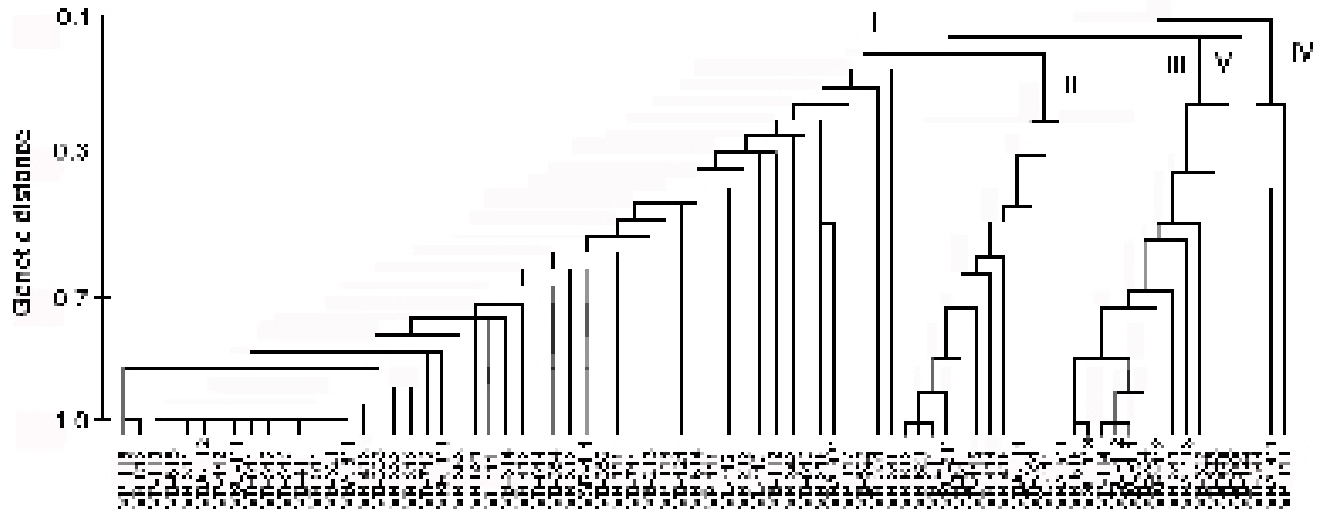

Figure 2. Dendogram based on UPGMA joining from Jaccard similarity matrix on 78 accessions of Amorphophallus variabilis from Java, Indonesia. Code of accessions referred to Table 1

A. variabilis reproduces by using cormels and seed. Regarding the genetic diversity, it is likely linked to seed propagation. Seeds were mostly from cross pollination; even some degree of inbreeding might still occur (Santosa *et al*., 2004). The cross pollination presumably created genetic diversity within site and among closes *A. variabilis* populations. Understanding the structure of genetic population is important in order to study responsible factor that contributes to genetic variability.

It was also hypothesized that bird and rats might have assisted long seed dispersal. Naturally, seeds would disperse within area less than 2 m from the parent plant. Bright red-berries and their sweet taste pulp might attract birds and the rats. Another possible contributor to the variability might be by human dispersal. Since *A. variabilis* produced unpleasant odor (Sugiyama and Santosa, 2008), and exhibited snake like color pattern of petiole (Santosa *et al*., 2004), people might get ridge of this plant from their lands and dump them to other sites.

Furthermore, at time of the World War II in 1940s, local government ordered to farmers in Java to collect *Amorphophallus* corms for war logistic. The corms from eastern Java were collected and transported to western Java, particularly to Jakarta Port. Propagule mobilization during the world war might contributes to present genetic diversity. Nevertheless, there was lack of scientific report explaining propagules mobilization. Therefore, development of marker specific with high rate of mutation such as SSR (Lian *et al*., 2006) and lower rate of mutation such as FLORICAULA/ LEAFY (Grob *et al*, 2004) probably would valuable to trace whether or not mobilization among sites occurred in the past time.

Confronting with Morphological Characters

Comparison of AFLP and morphological grouping (Santosa *et al*., 2004) was performed for 52 accessions, both studies used different accessions. Number of matching group was low, particularly of Group I AFLP. Group I AFLP separated into four clusters at morphological grouping (or 7 sub-groups) (Table 3). Several morphological characters such as flower size, leaf size and absence of petiole spot linked tightly with AFLP grouping at Group II and Group III. Most accessions from Group II AFLP belong to cluster I of morphological characters except accessions from site 16 (VB-612-Cluster II), site 17 (VB 621-Cluster II), and from site 4 (VKU-211-Cluster III). Most accessions belonging to Group II have bigger flower size than those of other groups (Table 4).

All accessions belong to Group III were a member of Cluster I based on morphological classification. All of these accession have long leaflets and spathe, i.e., 14.0±2.3 and 14.2±2.7 cm (mean±SD), respectively. Three accessions (VP-114, VB-334 and VB-341), with absence of dot on petiole, belonged to Group III of AFLP. Furthermore, accessions belonging to AFLP Group IV and Group V are clustered into cluster IV based on morphological characters.

Table 3. Group membership based on cluster analysis of AFLP markers of 78 accessions of *A. variabilis*

District	Group					Total
	I	II	III	IV	V	
Bogor	35	7	6	1	1	50
Lebak	3					3
Pandeglang	6	1	1	1		9
Cianjur		1				1
Kuningan		1	1			2
Sukabumi	1		2			3
Banjarnegara	3	2	1	1		7
Karanganyar	1		1			2
Sragen	1					1
Total	50	12	12	3	1	

Table 4. Grouping of accessions based on AFLP and morphological characteristics [X]

District	I[Z]							II			III		IV	V
	A	B	C	D	E	F	G	B	F	D	B	C	A	G[Y]
Bogor	4	9	2	4	2	2	1	3	2		4	1		1
Lebak	2	1												
Pandeglang	1	2	1			1		1			1		1	
Sukabumi						1								
Cianjur								1						
Kuningan										1				

Note: [Z] I, II, III, IV, and V are groups based on AFLP markers in present study
[Y] A, B, C, D, E, F and G are groups based on morphological characters by Santosa *et al*. (2004)
[X] Comparison was carried out on 52 accessions, due to AFLP and morphological analyses were conducted at different number of accessions

CONCLUSIONS

AFLP markers were able to generate polymorphisms in accessions of *A. variabilis* from Java. The accessions showed high diversity and separated into 5 groups. Most of the grouping seemed independent of geographical origin. Most accessions with big flower size were in Group II, while those with long leaflet and spathe, and had no dot on petiole were in Group III. Accessions grouped into AFLP Group IV and Group V were clustered into Cluster IV based on morphological grouping. In order to increase genetic diversity, crossing among distinct groups should be considered. This experiment implies for *A. variabilis* conservation program, Bogor and Pandeglang districts could be selected as conservation regions.

ACKNOWLEDGEMENT

Part of this work was supported in part by a Grant-in-Aid from the Japan Society for the Promotion of Science.

REFERENCES

Grob, G.B.J., B. Gravendeel, M.C.M. Eurlings. 2004. Potential phylogenetic utility of the nuclear *FLORICAULA/LEAFY* second intron: comparison with three chloroplast DNA regions in *Amorphophallus* (Araceae). Mol. Phylogenet. Evol. 30:13-23.

Hetterscheid, W., S. Ittenbach. 1996. Everything you always wanted to know about *Amorphophallus*, but were afraid to stinks your nose into. Aroideana 19:7-131.

Huh, M.K., O. Ohnishi. 2002. Genetic diversity and genetic relationships of East Asian natural populations of wild radish revealed by AFLP. Breeding Sci. 52:79-88.

Jansen, P.C.M., C. van der Wilk, W.L.A. Hetterscheid. 1996. *Amorphophallus* Blume ex Decaisne. *In* M. Flach, F. Rumawas (*Eds*.). PROSEA 9: Plant Yielding Non-seed Carbohydrates. Backhuys Publ. Leiden.

Lian, C.L., M.A. Wadud, Q.F. Geng, K. Shimatani, T. Hogetsu. 2006. An improved technique for isolating codominant compound microsatellite markers. J. Plant Res. 119:415-417.

Loh, J.P., R. Kiew, A. Kee, L. H. Gan, Y. Y. Gan. 1999. Amplified fragment length polymorphism (AFLP) provides molecular markers for the identification of *Caladium bicolor* cultivars. Ann. Bot. 84:155-161.

Loh, P. J., R. Kiew, A. Hay, A. Kee, L. H. Gan, Y. Y. Gan. 2000. Intergeneric and interspecific relationships in *Araceae* tribe *Caladieae* and development of molecular markers using Amplified Fragment Length Polymorphism (AFLP). Ann. Bot. 85:371-378.

Mignouna, H. D., R. Mank , T. H.N. Ellis , N. van den Bosch, R. Asiedu, S. Ng, J. Peleman. 2002. A genetic linkage map of Guinea yam (*Dioscorea rotunda* Poir.) based on AFLP markers. Theor. Appl. Genet. 105:716-725.

Nishizawa, T., T. Kawahara, E. Kinoshita, K. Ueda, Y. Watano. 2003. Development of polymorphic microsatellite markers in *Arisaema serratum* (Thunb.) Schott, Araceae. Mol. Ecol. Notes 3:32-34.

Ohtsuki, T. 1968. Studies on reserve carbohydrates of four *Amorphophallus* species, with special reference to mannan. Bot. Mag. Tokyo 81:119-126.

Purba, A.R., J.L. Noyer, L. Baudouin, X. Perrier, S. Hamon, P.J.L. Lagoda. 2000. A new aspect of genetic diversity of Indonesian oil palm (*Elaeis guineensis* Jacq.) revealed by isoenzyme and AFLP markers and its consequences for breeding. Theor. Appl. Genet. 101:956-961.

Roa, A.C., M.M. Maya, C. Duque, J. Tohme, A.C. Allen, M.W. Bonierbale. 1997. AFLP analysis of relationships among cassava and other *Manihot* species. Theor. Appl. Genet. 95:741-750.

Rohlf, F.J. 2005. NTSYSpc Numerical taxonomy and multivariate analysis system ver. 2.1. Exeter Software. Applied Biostatistics Inc., New York.

Santosa, E., N. Sugiyama, S. Hikosaka, T. Takano. 2004. Classification of *Amorphophallus variabilis* in West Java, Indonesia based on morphological characteristics of inflorescences. Jpn. J. Trop. Agric. 48:25-34.

Schnell, R.J., R. Goenaga, C. T. Olano. 1999. Genetic similarities among cocoyam cultivars based on randomly amplified polymorphic DNA (RAPD) analysis. Sci. Hort. 80:267-276.

Steiger, D., C. Nagai, P. Moore, C. Morden, R. Osgood, R. Ming. 2002. AFLP analysis of genetic diversity within and among *Coffea arabica* cultivars. Theor. Appl. Genet. 105:209-215.

Sugiyama, N., E. Santosa. 2008. Edible *Amorphophallus* in Indonesia-Potential Crops in Agroforestry. Gajah Mada University Press, Yogyakarta. Indonesia.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, M. Zabeu. 1995. AFLP: a new technique for DNA fingerprint. Nucleid Acids Res. 23:4407-4414.

Widjaja, E.A., R.N. Lester. 1987. Morphological, anatomical and chemical analyses of *Amorphophallus paeoniifolius* and related taxa. Reinwardtia 10:271-280.

Yuzammi. 2000. A taxonomic revision of the terrestrial and aquatic Aroid (Araceae) in Java. Thesis in School of Biological Science, Faculty of Life Science, University of New South Wales, Australia.