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GENETIC DIVERSITY OF MADURESE BAMBARA GROUNDNUT (*Vigna subterranea* L. Verdc.) LINES BASED ON MORPHOLOGICAL AND RAPD MARKERS

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SUMMARY

Comparison of different methods in the calculation of genetic diversity can be useful in plant breeding and conservation programs. This study used twelve potential bambara groundnut lines. A total of 41 morphological characters and 10 RAPD primers were used to assess the genetic relationships based on the morphological characters and RAPD markers and to determine the potential lines of bambara groundnut with the desired traits for line development in plant breeding and conservation programs. Cluster analysis based on the similarity coefficient of 12 bambara groundnut lines using both morphological and RAPD markers produced 2 clusters with a similarity degree of 52% for morphological markers and 51% for RAPD markers. There were differences between morphological and RAPD dendrograms. DNA markers derived from RAPD analysis were unrelated to morphological characters. Therefore, the selection of Bambara groundnut could not be conducted on the basis of the difference in morphological characters.

Key words: Bambara groundnuts, morphological markers, RAPD markers

Key findings: The genetic relationships among local Madurese lines (G8, G9, and G10) had similarity coefficients of 0.83 to 0.94. Three potential bambara groundnut lines (G5, G9, and G11) for development in breeding and cultivation programs were obtained.

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INTRODUCTION

The Bambara groundnut *(Vigna subterranea* L.Verdc*.)* is a legume plant that originated from Africa. This crop has the potential to be one of the future alternative food sources since its dry seed contains 16–21% protein, 50–60% carbohydrate, 4.5–6.6% fat, calcium, phosphorus, iron, and vitamin B1 (Suwanprasert *et al*., 2006). This crop could also be developed for dry land areas, such as Madura. Bambara groundnut has the advantage of being able to survive in a dry environments with low nutrient content (Goli, 1995; Zeven, 1998; Berchie *et al*., 2012).

Bambara groundnut has not been widely developed in Indonesia. In East Java, it is only cultivated in Gresik, Lamongan, and Bangkalan Madura. This crop gains less attention in Indonesia and the productivity at the farm level remains low. A study by Redjeki (2003) showed that planting seeds of various colors produces 0.7 to 2.0 tons per hectare of dry seeds. This crop is capable of producing 4.0 tons per hectare of dry grains at optimal growing conditions (Kouassi and Zoro-Bi, 2010).

One of the causes of low productivity of bambara groundnut at the farm level is the use of local lines. Kuswanto *et al.* (2011) showed that among the 50 tested local lines of bambara groundnut originated from East Java and West Java, there were high levels of diversity in both within and between the lines, including the character of plant growth habit, leaf shape, and stem hairiness. Hence, plant breeding activities should be done immediately to improve the local lines by purifying the potential local lines followed by selection. Furthermore, these lines are expected to be developed into new varieties or to serve as parents in crossbreeding.

Development of the new varieties requires information on the genetic relationships among the materials serving as the prospective parents. Information on genetic relationships based on both morphological and molecular characteristics are required to select parents for breeding programs (Pabendon *et al*., 2007). The genetic relationships are then used to determine the genetic diversity and genetic distance among the genotypes tested. Information on genetic diversity could be obtained based on morphological characters both quantitative and qualitative (Collinson *et al.*, 1999; Massawe, 2000). Morphological characters have weaknesses because the characters are often influenced by environmental factors (Hadiati and Sukmadjaja, 2002; Massawe *et al*., 2003; Amzeri, 2015). Therefore, identification at the molecular level is required to complement the morphological characters (Galvan *et al*., 2001).

Identification at the molecular level is a highly effective method for analyzing plant genomes (Yuwono, 2006). One of the molecular markers for identification at the DNA level is the RAPD (random amplified polymorphic DNA) method. The RAPD method effectively and rapidly detects polymorphisms at some loci (Azrai, 2005). According to Tingey *et al.* (1994), the advantage of RAPD method is it does not require to have knowledge of the genome of the plant. RAPD markers have been widely used for tropical plants, especially for Araceae species (Irwin *et al*., 1998; Jimenez *et al*, 2002;

Prana and Hartati 2003; Nowbuth *et al*., 2005; Poerba and Yuzammi, 2008).

The objectives of this study were: (1) to examine the genetic relationships among bambara groundnut lines based on the morphological and RAPD characters, and (2) to determine the genotypes of bambara groundnut lines with potential characters for development in breeding and cultivation programs.

MATERIALS AND METHODS

Genetic materials

The study was conducted at Pagesangan village, Surabaya-East Java, Indonesia with altitude of 3-6 m above sea level, from December of 2016 until May of 2017. The genetic materials used were 11 potential lines of bambara groundnut derived from purification of local lines originating from various regions in Indonesia and one reference line from the collection of the Breeding Laboratory, Faculty of Agriculture, Brawijaya University Malang (UB Cream) (Table 1). Twelve potential lines under study resulted from the four cyles of selection of inbred lines on the basis of similarity of characters and high yield among the 158 bambara groundnut genotypes of Indonesia.

Morphological characterization

Morphological characterization was observed by planting 12 potential lines in a pot using grumosol soil during the wet season with an average rainfall of 100.7 to 465.7 mm and 7 to25 days of rain (Indonesian Agency for Meteorological, Climatological and Geophysics, Juanda Sidoarjo, East Java, Indonesia). The morphological characterization was done in a completely randomized design (CRD) replicated three times.

The morphological characters observed were quantitative and qualitative characters. The quantitative characters were sprout age (SA), first leaf (FL), flowering age (FA), number of harvest days (NHD), number of leave (NL), number of flowers (NF), plant height (PH), plant diameter (PD), leaf thickness (LT), terminal leaf width (TLW), terminal leaf length (TLL), internode length (IL), petiole length (PL), number of branches (NB), root length (RL), fresh weight of crop residues (FWtC), number of stem segments (NSS), number of pods (NP), fresh weight of pods per plant (FWtP), fresh weight of pod per pod (FWtPP), dry weight of crop residues (DWtC), dry weight of pod per plant (DWtP), pod length (PL), pod width (PW), skin weight (SW), number of seeds (NS), seed weight per plant (SWP), and seed weight per seed (SWS). While the qualitative characters were hypocothyl pigment, hypocothyl groove, color of terminal leaflet, terminal leaflet shape, growth habit, pigmentation on wing, stem hairiness, pod color, pod shape, pot texture, two seed pods, seed color, and seed shape.

Isolation of DNA

DNA was isolated using CTAB method, in which 0.1 g sample of fresh leaves was crushed in a mortar. A total of 1500 μl of CTAB buffer solution (consisting of 2% CTAB, 1.4 M NaCl, 100 mM Tris HCl pH 8, 20 mM EDTA pH 8, 2% PVP-40 1, and 2% mercaptoethanol), previously incubated on a water bath

Table 1. Code, name and origin of bambara groundnut lines studied.

No.	Code	Name of Line	Origin of Line
1	G1	GSG 1.1.1	Gresik, East Java, Indonesia
2	G2	GSG 2.4	Gresik, East Java, Indonesia
3	G3	GSG 3.1.2	Gresik, East Java, Indonesia
4	G4	BBL 2.1.1	Lamongan, East Java, Indonesia
5	G5	BBL 6.1.1	Lamongan, East Java, Indonesia
6	G6	CCC 2.1.1	Cianjur, West Java, Indonesia
7	G7	GSG 2.1.1	Gresik, East Java, Indonesia
8	G8	JLB 1	Madura, East Java, Indonesia
9	G9	CKB 1	Madura, East Java, Indonesia
10	G10	TKB 1	Madura, East Java, Indonesia
11	G11	PWBG 5.3.1	Gresik, East Java, Indonesia
12	G12	UB Cream	UB (University of Brawijaya), East Java, Indonesia

at 65°C for 30 min, was added. The crushed mixture was subsequently incubated at 65°C for 60 min. The mixture was stirred for 10 min to maintain homogeneity.

After incubation, the mixture was taken from the water bath and left for 2 minutes. Subsequently, 500 µl of chloroform-isoamyl alcohol (CIAA) (24:1) were added to each sample and subjected to a vortex for 5 min and to centrifugation for 15 min at 12,000 rpm. The supernatant was taken carefully and sodium acetate at a 1/10 of the supernatant volume was added. Cold isopropanol of 2/3 of the total (supernatant + sodium acetate) volume was then added and mixed by shaking the tube and left at 4°C for 1 to 24 hours. Furthermore, the supernatant was centrifuged at 12,000 rpm for 10 min. It was then removed and the precipitated DNA was washed with 500 µl of 70% ethanol and centrifuged for 5 min at 12,000 rpm. The supernatant was removed and the precipitated DNA was dried. After drying, the precipitated DNA was dissolved with 50 µl of ddH_2O (distilled) solution and then stored in a refrigerator at 4°C. The quantity of the extracted DNA was tested using *Gene Quant* and then measured for absorbance at a wavelength of 260 nm.

PCR-RAPD analysis

DNA amplification was performed using the Bio-Rad PCR system with a total volume of 10 µl for each PCR tube. Each PCR reaction consisted of 5 µl of Go Taq® Green (Promega) PCR mix, 0.25 µl of 100 µM primer (OPA-4, OPA-5, OPA-9, OPB-15, OPC-2, OPD-3, OPD-13, OPD-15, OPD-20, and OPC-1), 2.5 µl of DNA sample (template), and 2.25 nuclease-free water. The first heating was carried out at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 37°C for 30 s, and elongation at 72°C for 1 min and 30 s, and then followed by final elongation at 72°C for 5 min.

The PCR-derived DNA was subsequently electrophoresed using 1.5% (w/v) agarose, previously added with FloroSafe DNA Stain as a

dye, in a TBE buffer (consisting of 0.45 M of Tris-HCl pH 8, 0.45 M of Boric acid, and 20 mM of EDTA) at a voltage of 100 volts for 45 min. The results were visualized under UV light.

Data analysis

The genetic relationships were determined on the basis of morphological character by using the Pearson's correlation formula and characterization and assessment in a *n* (morphological character) x *t* (operational taxonomic unit) table. The operational taxonomic unit is the characterized bambara groundnut lines. Scoring consisted of the binary scoring for the presence or absence of a trait and the multi-state scoring for the qualitative and quantitative traits. Standardization of traits was carried out to process data into a binary data. The similarity matrix was then subjected to a cluster analysis. A dendrogram was constructed from the formed clusters using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method. Then, cophenetic correlation coefficient between the similarity matrix and dendrogram was calculated. The similarity coefficient indicates the accuracy of clustering. The entire analysis was performed using the NTSys version 2.1 software (Rohlf, 2000).

The RAPD marker-based genetic relationships were based on whether or not DNA bands are present. The DNA band profile was translated into a binary data which 0 indicates no DNA band and 1 indicates presence of DNA bands at the same position of the individual being compared. Cluster analysis and dendrogram were made using the unweighted pair group method with arithmetic mean (UPGMA) method with the numerical taxonomy and multivariate system (NTSYS) version 2.1 software.

RESULTS AND DISCUSSION

Diversity analysis based on morphological characters

The results of clustering based on morphological characters of bambara groundnut lines do not always provide clustering by region of origin, but by the similarity of morphological characters among bambara groundnut lines tested (Tables 2 and 3). Genetic sequencing and selection lead to a greater genetic diversity than plant-growing distances; thus, despite the same region of origin of bambara groundnut genotypes, different growing environments may affect genetic diversity (Akmal, 2008). Furthermore, according to Jose *et al*. (2005), genotypes from the same region of origin do not always belong to the same cluster. The results of this study are in line with the study done by Yang *et al.* (2006) showing that bambara groundnut was not clustered on the basis of its origin. In the assessment of the closeness of genetic relationships, genotype clustering is determined by the similarity of morphological characters.

Table 2. Qualitative characters performance of Madurese bambara groundnut lines.

Character	Genotypes											
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
Hypocotyl pigment	Yellowish Green	Yellowish Green	Green	Green	Green	Yellowish Green	Yellowish Green	Yellowish Green	Green	Green	Green	Red
Hypocotyl groove	present	Present	present	present	present	absent	Present	present	present	absent	present	present
Color of terminal leaflet	Bright Green	Bright Green	Bright Green	Bright Green	Bright Green	Dark Green	Bright Green	Bright Green	Dark Green	Dark Green	Bright Green	Bright Green
Terminal leaflet shape	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Elliptical	Lanceolate	Lanceolate	Elliptical	Elliptical	Lanceolate	Lanceolate
Growth habit	Spreading	Spreading	Semi-Bunch	Semi-Bunch	Spreading	Semi-Bunch	Semi-Bunch	Semi-Bunch	Spreading	Spreading	Spreading	Semi-Bunch
Flower pigment	present	Present	present	present	present	present	Present	present	present	present	present	present
Stem hairiness	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Pod color	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Dark	Bright
Pod shape	Pointed	Pointed	Pointless	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed
Pod texture	Rough	Rough	Rough	Rough	Rough	Rough	Rough	Rough	Rough	Rough	Rough	Smooth
Two seed pods	absent	Present	present	present	present	absent	Absent	absent	present	present	present	absent
Seed color	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Cream
Seed shape	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval

Table 3. Quantitative characters of Madurese bambara groundnut lines.

Character	Genotype											
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
SA	7.33	6.67	6.67	6.33	6.33	5.67	7.67	7.33	6.33	7.33	6.67	8.00
FL	10.00	8.33	10.00	8.67	8.00	9.00	10.00	10.00	10.33	9.33	9.33	9.67
FA	40.67	39.67	40.67	38.33	39.33	42.00	44.67	38.33	43.33	46.00	41.00	48.33
NHD	139.67	132.33	135.00	132.00	134.67	131.00	137.33	134.67	138.33	127.00	136.00	153.33
NL	87.67	93.33	93.67	98.33	119.67	86.33	87.33	91.00	104.33	97.33	150.67	141.00
NF	176.00	246.33	173.67	190.67	292.00	144.33	157.33	221.00	143.67	155.33	268.67	563.33
PH	28.00	28.67	24.33	30.67	26.00	28.67	25.33	30.67	25.00	25.33	27.33	31.67
PD	57.00	57.67	52.67	60.00	57.67	52.67	51.33	62.33	54.67	51.33	59.33	69.67
LT	0.14	0.14	0.17	0.11	0.11	0.10	0.10	0.18	0.10	0.19	0.12	0.15
TLW	2.59	2.37	2.43	2.59	2.30	3.20	2.19	2.69	3.11	3.07	2.43	3.03
TLL	8.56	8.66	7.87	8.67	7.42	7.53	7.57	8.64	6.94	6.76	7.27	8.86
IL	2.96	2.60	2.48	2.54	2.89	2.67	2.52	2.81	2.61	2.32	2.79	3.53
PL	18.23	17.94	16.39	18.60	17.70	19.13	17.54	19.66	17.39	17.04	16.76	25.07
NB	7.00	7.00	9.33	8.67	7.33	8.33	6.33	7.00	9.00	9.67	9.00	8.00
RL	14.17	15.27	12.77	13.70	13.00	10.73	12.17	14.40	14.50	12.50	12.60	14.00
FWtC	51.33	60.67	60.67	86.00	79.00	53.33	50.00	83.67	63.33	65.33	103.00	166.33
NSS	8.22	8.56	9.00	8.22	8.78	6.67	8.22	9.22	8.00	7.44	9.78	18.33
NP	42.67	67.33	71.67	51.67	71.33	46.33	53.67	49.33	86.00	68.67	83.33	29.67
FWtP	55.00	84.33	82.67	90.00	106.33	98.00	80.00	60.00	120.67	71.33	104.00	53.33
FWtPP	1.29	1.25	1.15	1.74	1.49	2.12	1.49	1.22	1.40	1.04	1.25	1.80
DWtP	24.27	41.27	38.80	38.47	45.73	39.87	35.33	31.10	54.43	33.50	47.03	23.13
DWtC	24.17	26.27	25.57	28.13	29.73	20.67	21.80	30.30	25.93	22.37	36.87	87.63
PL	16.75	18.07	18.76	19.39	18.56	20.69	17.83	18.32	18.22	16.54	17.74	21.19
PW	13.49	13.96	15.11	15.61	14.81	19.02	14.35	14.51	14.87	13.21	14.71	14.93
SW	4.24	10.22	8.28	8.52	9.52	10.42	8.03	6.06	11.59	6.45	9.64	6.29
NS	42.00	63.00	70.67	52.67	72.67	41.33	55.00	53.33	91.33	68.67	78.67	25.67
SWP	20.03	31.04	30.52	29.95	36.21	29.44	27.30	25.04	42.85	27.05	37.39	16.85
SWS	0.45	0.47	0.43	0.55	0.50	0.73	0.50	0.47	0.47	0.39	0.47	0.62

Notes: sprout sge (SA); first leaf (FL); flowering age (FA); number of harvest days (NHD); number of leaves (NL); number of flowers (NF); plant height (PH); plant diameter (PD); leaf thickness (LT); terminal leaf width (TLW); terminal leaf length (TLL); internode length (IL); petiole length (PL); number of branches (NB); root length (RL); fresh weight of crop residues (FWtC); number of stem segments (NSS); number of pods (NP); fresh weight of pods per plant (FWtP); fresh weight of pod per pod (FWtPP); dry weight of crop residues (DWtC); dry weight of pod per plant (DWtP); pod length (PL); pod width (PW); skin weight (SW); number of seeds (NS); seed weight per plant (SWP); seed weight per seed (SWS).

According to de Zousa (2008), the more morphological similarities among the genotypes tested, the closer the genetic relationships are.

Clustering based on morphological characters produced a dendrogram with similarity coefficients of 0.33 to 1.00 (Figure 1). A high similarity coefficient indicates that those lines had a close genetic relationship. At a similarity coefficient of 0.52 there were two main clusters, Cluster I and Cluster II. The characters distinguishing Cluster I from Cluster II were hypocotyl pigment color, pod color, and seed color. The reference line of Cluster I had red hypocotyl pigments, bright pods, and cream seeds. The lines in Group II had green and yellowish-green hypocotyl pigments, dark pods, and purplish black seeds. The color of hypocotyl pigments, pod color, and seed color are the characters that can be used to distinguish and compare the clusters (cluster I) from potential line under study (cluster II). Group II formed two sub-clusters: Cluster A and Cluster B. Cluster A and Cluster B diverged due to their differences in leaf color and shape. Cluster A had dark green leaves and elliptical leaves, while Cluster B had bright green leaves and lanceolate leaves. The clustering of lines was not based on the region from which the lines originated but on morphological similarity of the lines studied. The same results also reported by Wicaksana *et al*. (2013) based on PCA analysis on qualitative and quantitative characters of lines of Bandung, Garut, Lamongan, Bogor, Majalengka, Madura, Sumedang, and Tasikmalaya. This may have resulted from the easiness of tranporting the plants to any producing centres.

Observation and measurement of the 12 lines of bambara groundnut showed three lines with a high wet weight of pods per plant: G5, G9, and G11. All three lines can be used as parents to develop varieties with high yield. According to Halluer *et al*. (2010), the parents selected for breeding and cultivation development programs, in addition to having high yield, should have a good combining ability and a distant genetic relationship in order to avoid inbreeding depression. The results of Baskorowati (2017) showed that antogamy of *Melalueca alternifolia* resulted in inbreeding depression indicated by low seed production, low germination, and slow seedling growth. G5 and G11 had a similarity coefficient of 1.00, meaning that both lines are a single line, despite their different origin. There was no change in characters in the two lines (G5 and G11), although they were planted in different places.

Diversity analysis based on molecular RAPD marker

Amplification of the ten primers generated 84 bands from the 12 bambara groundnut lines tested (Figure 2). There were various number of bands ranging from 5 bands (OPD 15) to 13 bands (OPD 13), while the percentage of polymorphism ranged from approximately 20% (OPB 15) to 100% (OPD 3, OPD 15, and OPC 1) and the average percentage of polymorphic band was 82.14% (Table 4). A study by Masawe *et al*. (2003) showed that the tests of 12 bambara groundnut genotypes using 16 primers produced polymorphic bands with an average percentage of 73.10%.

Table 4. Number of polymorphic loci of ten RAPD primers applied on Madurese bambara groundnut lines.

No	Primer	Base Sequence (5'-3')	Number of Amplified Band	Monomorphic Band	Polymorphic Band	Percentage of Polymorphic Band
1	OPA 4	AATCGGGCTG	7	2	5	71.43
2	OPA 5	AGGGGTCTTG	7	1	6	85.71
3	OPA 9	GGGTAACGCC	9	2	7	77.78
4	OPB 15	GGAGGGTGTT	5	4	1	20.00
5	OPC 2	GTGAGGCGTC	11	2	9	81.82
6	OPD 3	GTCGCCGTCA	12	0	12	100.00
7	OPD 13	GGGGTGACGA	13	1	12	92.31
8	OPD 15	CATCCGTGCT	5	0	5	100.00
9	OPD 20	ACCCGGTCAC	7	3	4	57.14
10	OPC 1	TTCGAGCCAG	8	0	8	100.00
Total			84	15	69	82.14

Table 5. Similarity matrix of Madurese bambara groundnut lines using RAPD markers.

Genotip	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
G1	1.00											
G2	0.91	1.00										
G3	0.91	0.88	1.00									
G4	0.88	0.94	0.89	1.00								
G5	0.61	0.54	0.58	0.57	1.00							
G6	0.57	0.50	0.58	0.49	0.78	1.00						
G7	0.59	0.52	0.60	0.51	0.72	0.92	1.00					
G8	0.57	0.50	0.56	0.51	0.80	0.86	0.84	1.00				
G9	0.56	0.51	0.57	0.52	0.71	0.89	0.89	0.83	1.00			
G10	0.59	0.52	0.60	0.53	0.82	0.90	0.86	0.94	0.85	1.00		
G11	0.86	0.49	0.59	0.50	0.73	0.91	0.91	0.89	0.90	0.89	1.00	
G12	0.56	0.49	0.57	0.50	0.77	0.95	0.89	0.87	0.90	0.91	0.90	1.00

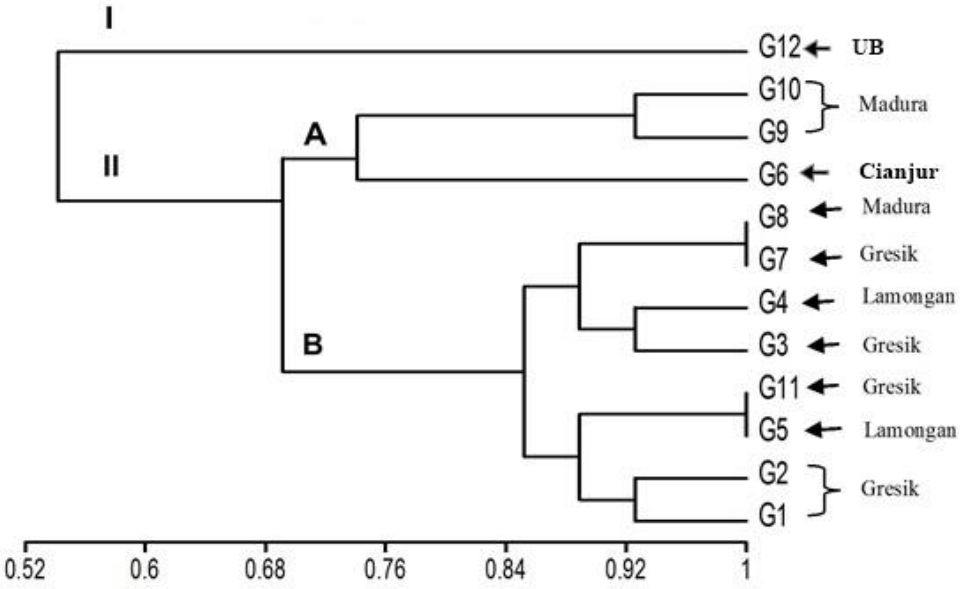

Figure 1. Dendrogram of 12 lines of bambara groundnut lines by morphological characters.

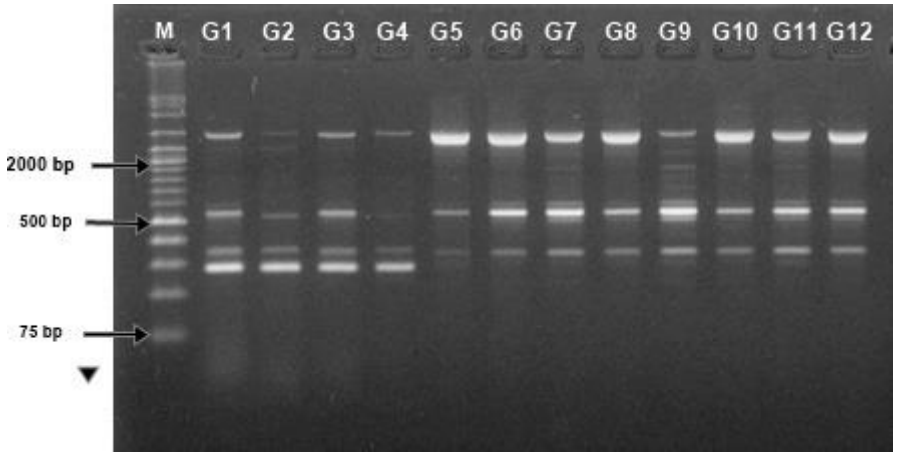

Figure 2. RAPD bands of twelve bambara groundnuts lines with OPD 20 primers.

The RAPD marker of bambara groundnut lines showed that G6 and G12 had the closest genetic relationship with a similarity coefficient of 0.95, followed by G2, G4, G8, and G10 with a similarity coefficient of 0.94 (Table 5). G2, G11, and G12 had the most distant genetic relationship with a similarity coefficient of 0.49. The resulting cluster was different from that of bambara groundnut lines regarding morphological characters. The amplified bands did not indicate characteristics associated with morphological characters. The difference in the number and base sequence of the amplified DNA bands on the primer is highly instrumental in determining the level of genetic diversity. Saraswati *et al*. (2017) discovered that the number of polymorphic DNA bands could describe the genome profiles of bambara groundnut plants since they could indicate the distribution of primer attachment sites on the genomes.

The similarity level of 0.51 formed two main clusters: Cluster I and Cluster II. Cluster II formed two sub-clusters: Cluster A consisting of 1 line (G5) and Cluster B consisting of 7 lines. The RAPD marker was capable of clustering lines originating from Madura (G9 and G10) at a similarity coefficient of 0.85. The clustering formed by the two lines was in line with the morphological character-based clustering.

The RAPD marker-based dendrogram did not provide an obvious clustering of bambara groundnut lines. This was due to the highly polymorphic amplified bands (84%), preventing it from obtaining specific bands capable of obvious clustering. The low similarity among the band patterns of bambara groundnut lines lead the RAPD data to have a low justification for their genetic relationship but it is appropriate to find variability among the bambara groundnut lines to determine the candidates for superior parents.

A breeding program to obtain the desired varieties through crossing requires the formation of segregating populations. The selected candidate parents should have a large degree of diversity. The dendrogram shows that the genetic relationship among local Madurese lines (G8, G9, and G10) had a high similarity coefficient of 0.83 to 0.94 (Figure 3). This makes breeding programs by hybridization difficult to produce the desired superior varieties due to inability to generate segregating population with high genetic variation.

The local Madurese bambara lines can be used as parents when they are crossed with other local lines from different clusters, i.e. G1, G2, G3, and G4. Bambara lines in Cluster I had a genetic distance from local Madura lines ranging from 0.50 to 0.60. Crosses between local Madurese lines and those lines in Cluster A would result in a large segregation, making it easier to select the desired varieties in segregating populations.

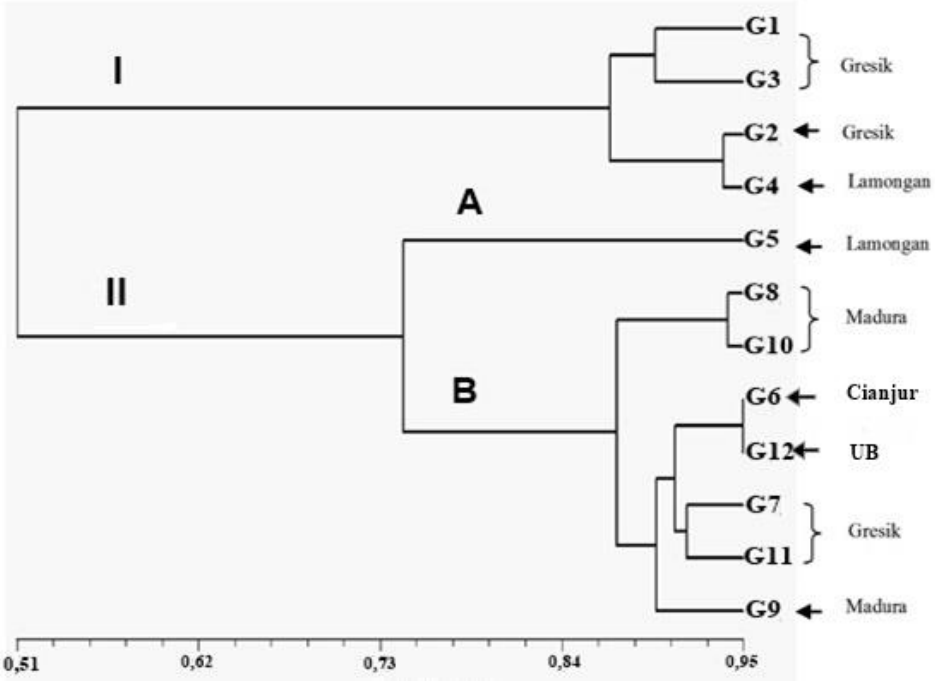

Figure 3. Dendrogram of 12 potential bambara groundnut lines based on RAPD marker.

CONCLUSION

Cluster analysis based on the similarity coefficient of 12 bambara groundnut lines using morphological and RAPD markers produced 2 clusters with a degree of morphological similarity of 52% for morphological markers and 51% for RAPD marker. There were differences between morphological and RAPD dendrograms, where the DNA bands derived from RAPD analysis were unrelated to morphological characters. Therefore, selection of bambara groundnut could not be conducted on the basis of the differences in morphological characters. The genetic relationships among local Madurese lines (G8, G9, and G10) had a high similarity coefficient of 0.83 to 0.94. Three bambara groundnut lines (G5, G9, and G11) were found as potential lines for development in breeding and cultivation programs.

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