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Genetic variability of Indonesian landraces of *Vigna subterranea*: Morphological characteristics and molecular analysis using SSR markers

MUHAMMAD FAUZAN FARID AL HAMDI[1], ASEP SETIAWAN[2], SATRIYAS ILYAS[2,♥], WAI KUAN HO[3,4]
[1]Graduate School, Institut Pertanian Bogor. Jl. Lingkar Akademik, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia
[2]Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8629353, ♥email: satriyas252@gmail.com
[3]Biotechnology and Crop Genetics Theme, Crops For the Future. Jl. Broga, 43500 Semenyih, Selangor, Malaysia
[4]School of Biosciences, Faculty of Science, University of Nottingham Malaysia Campus. Jl. Broga, 43500 Semenyih, Selangor, Malaysia

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Abstract. *Al Hamdi MFF, Setiawan A, Ilyas S, Ho WK. 2020. Genetic variability of Indonesian landraces of Vigna subterranea: The morphological characteristics and molecular analysis using SSR markers. Biodiversitas 21: 3929-3937.* Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is a potential grain, which can be considered as an alternative source of protein and carbohydrate. Due to unavailability of commercial bambara groundut cultivar in Indonesia, the characterization of bambara groundnut landraces is an important step before developing cultivar with traits of interest. The objective of the research was to access genetic variability of Indonesian landraces of bambara groundnut with different seed coat colors based on morphological and molecular markers. The experiment was arranged as split-plot in a complete randomized block design with the main plot was cultivation methods and the sub plot was landraces. There were differences in leaf shape and pod shape among the landraces. There were two main clusters of Indonesian landraces of bambara groundnut with 88.28% similarity. The first cluster was Cream, Brown Sumedang, Black Sumedang and Black Tasikmalaya, and the second cluster was Black Sukabumi, Brown Gresik, Black Madura, and Black Gresik. The result based on SSR marker with capillary electrophoresis indicated Black Gresik and Black Madura landraces were different from other Indonesian landraces. Cream Sumedang or Brown Sumedang from the first cluster and Black Gresik or Brown Gresik from the second cluster have the farthest distances for developing improved variety of bambara groundnut.

Keywords: Capillary electrophoresis, leaf shape, pod shape, simple sequence repeat, testa color

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is one of neglected crops in Indonesia and has potential to be developed as an alternative food source. It has a fairly high content of protein (16 - 21%), carbohydrate (50-60%), and low in fat (4.5-6.5%) (Suwanprasert et al. 2006). Bambara groundnut can be cultivated on marginal lands. According to Key (1979) bambara groundnut is tolerant to drought and poor soil conditions where peanut (*Arachis hypogaea* L.), corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench) can not well-survive in these conditions

Production of bambara groundnut in Indonesia was not optimal. According to Redjeki (2003), the average production of bambara groundnut in Indonesia was 2 tons ha^{-1} of the dry pod, while Kouassi and Zoro (2010) reported that high-density bambara groundnut cultivation in Ivory Coast could produce 3.9 tons ha^{-1}. Less production of bambara groundnut in Indonesia because of less information about cultivation method and still using the landraces instead of cultivar. Therefore, trying a variety of cultivation methods of bambara groundnut is still needed besides developing a high-yielding cultivar in Indonesia. The use of improved varieties suitable for the Indonesian environment and suitable cultivation method is expected to increase the production of bambara groundnut in Indonesia.

Bambara groundnut has the potential to replace soybean as an important legume in Indonesia because it is easily grown and drought tolerant. Bambara groundnut is still considered as a side crop by the Indonesian government, therefore, no cultivar has been developed yet. Due to the unavailability of cultivar, bambara groundnut farmers in Indonesia used landraces as planting material. Landraces have several disadvantages compared to commercial cultivars, such as non-uniform yield produced (Heller et al. 1997) and not as vigor as hybrids or other improved varieties (Mabhaudhi and Modi 2010). Therefore, purifying lines and characterization of Indonesian landraces are important before developing Bambara groundnut cultivar in Indonesia.

Morphological variability within Bambara groundnut landraces have been reported (Ntundu et al. 2006; Ouedrago et al. 2008; Siise and Massawe 2012; Austi et al. 2014). The result of Ntundu et al. (2006) showed 63 and 65% of the total variance among Tanzanian accessions during the two test seasons. While Siise and Massawe (2012) showed that the distance between Ghanaian landraces of bambara groundnut ranged from 70-92%, Austi et al. (2014) stated that bambara groundnut Indonesian landraces have narrow diversity. The high similarities among Indonesian landraces tested could be due to close kinship. However, morphological variability is not an accurate measure of diversity as it is affected by the growth stage of the plant and environmental conditions (Siise and Massawe 2012). Molecular marker analysis is not affected by these factors.

Simple sequence repeats (SSRs) or microsatellites are tandem repeated motifs that are one to six nucleotides in length and present in all prokaryotic and eukaryotic genomes (Zane et al. 2002). SSR markers offer several advantages including high polymorphic levels, specific loci, easy to reproduce, only need a small amount of DNA, scattered in the genome, high repeatability, and the most important is co-dominant (Kalia et al. 2011). SSR amplification result of Siise and Massawe (2012) showed 10 pairs of primers were polymorphic from screening 48. Agarose gel 2% could differentiate up to 50 base pairs (bp) of fragment length, but polyacrylamide gel could achieve higher concentration and hence had a higher resolution. Capillary electrophoresis, an electrophoretic separation carried out in capillary tubes, offers the possibility of fast and automatic analysis of small volume complex mixture with resolution and sensitivity (Gordon et al. 1988). A mix of amplified fragments can differentiate in single base-pair resolution through capillary gel electrophoresis (Applied Biosystem 2014). This research study aimed to access the genetic variability of Indonesian landraces of Bambara groundnut with different seed coat color based on morphological and molecular markers.

MATERIALS AND METHOD

Materials

A total of eight landraces varying in seed coat color was evaluated in this study. The seeds were originated from Madura (collection of Brawijaya University, Malang, Indonesia), Sumedang (farmer group), Gresik, Tasikmalaya, and Sukabumi (collection of IPB University, Bogor, Indonesia). Districts of Sumedang, Tasikmalaya and Sukabumi are located in West Java Province, while Districts of Gresik and Madura Island are located in East Java Province (Figure 1).

The seeds from Madura and Sumedang were harvested in March 2017 and May 2017, respectively, while the ones from Gresik, Tasikmalaya, and Sukabumi were harvested in May 2016. The seeds were sun-dried for two weeks before stored at 16°C until they were being used for the experiment. Sumedang landraces were then divided into Cream Sumedang, Brown Sumedang, Black Sumedang. Gresik landraces were divided into Black Gresik and Brown Gresik.

Methods

Field experiment was conducted in Mekarmulya Village, Situraja Sub-district, Sumedang District, West Java, Indonesia (6°51'09.4"S and 108°01'01.5"E) from August 2017 to January 2018. Planting was done on August 1st and harvesting was started on December 29, 2017. This experiment was arranged as split-plot in a randomized completely block design (RCBD) with two factors and three replicates. The first factor was landraces: Cream Sumedang, Brown Sumedang, Black Sumedang, Black Madura, Black Gresik, Brown Gresik, Black Tasikmalaya, and Black Sukabumi. The second factor was cultivation methods: the local farmer practice and the CFF protocol (a common protocol for the ITPGRFA-BSF Project). Local farmer's method used chicken manure and rice husk at a ratio of 2:1 with 2 ton ha^{-1} dosage at 2 weeks before planting, and the application of fertilizers [0.3 g urea (45% N), 1 g SP-36 (36% P_2O_5), 1 g KCl (60% K_2O)] on each plant hole at 5 weeks after planting, and the plant spacing was 25x50 cm. In CFF protocol, the same dosage of N, P and K fertilizer was applied twice, i.e. at sowing and 5 weeks after planting.

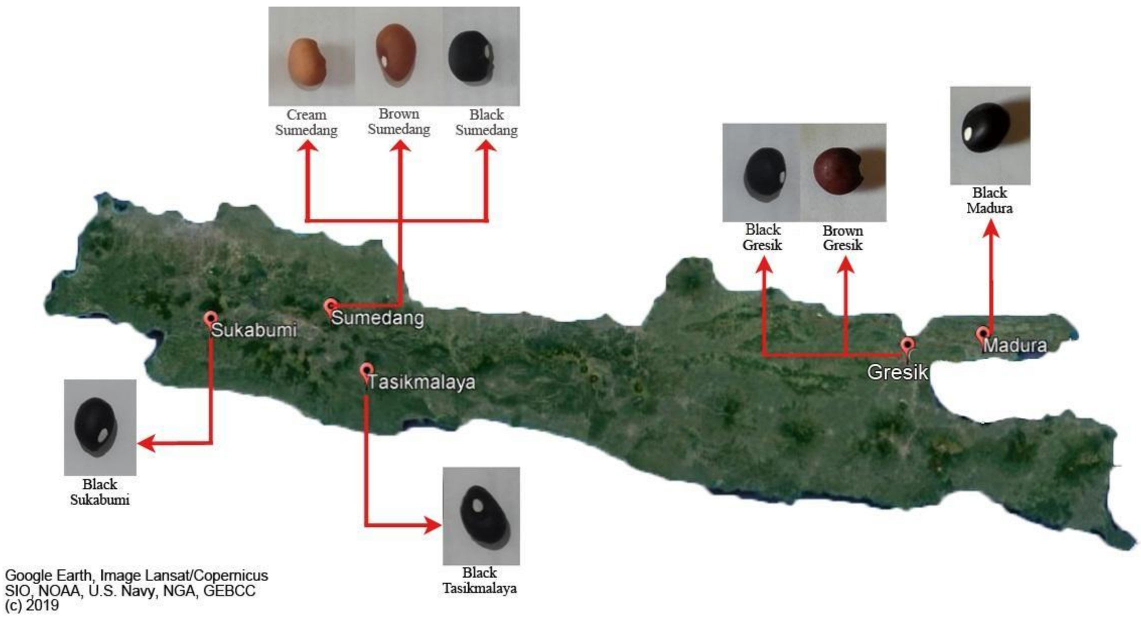

Figure 1. The source of eight Indonesian landraces of bambara groundnut

Morphological marker

Morphological characterization of eight Indonesian landraces of bambara groundnut was recorded dan described using bambara groundnut descriptor (IPGRI et al. 2000). The quantitative characters used as parameters were: days to emergence, plant height, leaf length, leaf width, number of leaves, canopy diameter, number of days to 50% flowering, number of pods per plant, seed length, and seed width. Plant height, middle leaflet length, middle leaflet width, number of leaves and canopy diameter were measured at 10 weeks after planting (WAP). Number of days to 50% flowering was recorded when 50% of the population per plot showed the first flower before analyzing the landrace. Number of pods was counted directly after harvesting. Seed length and seed width were measured after harvesting and sun-drying for one week. The qualitative characters were seed coat (testa) color, terminal leaf shape, and pod shape.

Molecular marker

A total of 24 seeds from eight landraces were sown. Leaves of the plant were cut (0.05 g leaf per sample) at 10 days after planting. Each landrace consisted of 3 replicates. DNA extraction was carried out using the CTAB (cetyl trimethyl ammonium bromide) method modified by Doyle and Doyle (1990) followed by PCR. PCR condition were set as follows: denaturation; 15 minutes; 94ºC, annealing 30 minutes; temperature according to Table 1, extension; 15 minutes; 94ºC and 30 minutes; 55ºC). PCR products were confirmed using 2% lithium borate agarose gel electrophoresis with SYBR™ Safe (Invitrogen) dye.

SSR amplification was done using three pairs of selected primers (SSR P33, SSR P37, and SSR D11) (Table 1), as described by Molosiwa et al. (2015). In order to analyze the length of the PCR products by capillary electrophoresis, primers of SSR 33 and SSR37 were labelled with 6-carboxy-fluorescine (FAM) while primer of SSR D-11 was labelled with hexachloro-6-carboxy-fluorescine (HEX). This was achieved by using three primer systems as described by Schuelke (2000). The PCR product separated by capillary electrophoresis was scored using Peak Scanner 2 according to user guide of Applied Biosystem (2014).

Data analysis

Morphological and yield characters were scored and analyzed using ANOVA performed by SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA). The significant data was then analyzed with LSD at 5% (Siise and Massawe 2012). The correlation between morphological characters was expressed on each node of the dendrogram using the software Minitab 16.

RESULT AND DISCUSSION

Effect of cultivation method on plant growth and yield

Cultivation methods of bambara groundnut had no significant effect on all morphological characters of the plants except for fresh pod weight per plant (Table 2). It showed that plant spacing and different fertilizer treatments used in both methods did not affect plant performances except on fresh pod weight per plant. The fresh weight of pod per plant (110.7 g) was significantly higher using Local farmer's method than CFF's method (82.7 g). It was allegedly caused by the chicken manure and rice husk. The soil treated with manure was found to be loose, which probably provided adequate aeration into the soil and improved soil microbial activities (Xiao et al. 2006). According to Hossain and Ishimine (2015), the soil without manure showed a waterlogging condition for some time and dried earlier as compared with the soil treated with manure, indicating that the soil without manure has lower porosity and farmyard manure improves water holding capacity of soil. Several studies (Whalen et al. 2003; Seobi et al. 2005) revealed that organic manure increases pH and water-holding capacity, and decreases bulk density in soil. The loose soil was probably favorable for root growth and pod formation. However, this has no significant difference in dry weight pod per plant and number of pod per plant.

Morphological characterization

There were three qualitative characters recorded in this study i.e. testa color, leaf shape, and pod shape. Three types of testa color were found after harvesting i.e. black, cream, and brown (Figure 3, Table 3). Both Black Tasikmalaya and Black Sukabumi produced 100% black testa color. Meanwhile, Sumedang and Gresik landraces were not consistent to produce the same testa color. Cream and Brown Sumedang tend to produce brown testa color while Black Sumedang, Black Gresik, Brown Gresik and Black Madura tend to produce black testa color.

Table 1. Name of the primers, their sequences, annealing temperature and size of the PCR product expected

Primer	Base	Annealing temperature (°C)	Size of the PCR product expected (bp)
P33 F	ACGCTTCTTCCCTCATCAGA	57	175
P33 R	TATGAATCCAGTGCGTGTGA		
P37 F	CCGATGGACGGGTAGATATG	55	256
P37 R	GCAACCCTCTTTTTCTGCAC		
D11 F	GAGGAAATAACCAAACAAACC	59	198
D11 R	CTTACGCTCATTTTAACCAGACCT		

The difference of testa color within landraces from same origins can be observed in Black Sumedang landrace and Brown/Cream Sumedang landrace. Cream and Brown Sumedang landraces were likely to be the same landrace with color shade difference could be due to premature harvest but Black Sumedang was almost consistent to produce seeds with black testa color (95% black and 5% brown). Both of Gresik and Madura landraces showed the same proportion of testa color (90% black, 10% brown) (Table 3). Gresik and Madura landraces were expected to have close kinship due to the proximity of their collection sites.

Austi et al. (2014) stated that the variability of 29 local landraces based on morphological markers was very low of 71-100% similarity. Despite the low variability, testa color differences observed have suggested a certain degree of genotypic differences among these landraces.

Black Sumedang landrace is also different from other Sumedang landraces in terms of leaf shape. Both Black Sumedang landrace and Black Tasikmalaya landrace had lanceolate in leaf shape (Figure 2). Cream and Brown Sumedang showed oval leaf shape, while Black Gresik, Brown Gresik, Black Madura, Black Tasikmalaya and Black Sukabumi showed elliptic leaf shape. Plants with lanceolate leaf shape in most cases are drought tolerant (Ghafoor et al. 2001), while wide leaf can produce more photosynthate to fill the pod (Sinuraya et al. 2015).

According to the descriptor of bambara groundnut (IPGRI et al. 2000), the pod shape of bambara groundnut can be classified into four categories (Figure 4). Five out of eight landraces had the pod shapes ending in a point, round on the other side (no. 2), while the pod shape of Black Gresik, Brown Gresik and Black Madura was ending in a point, with nook on the other side (no. 3). Interestingly, the pod shape of West Java origin (Sumedang, Sukabumi, and Tasikmalaya) was different from the ones that originated from East Java (Madura and Gresik) (Table 3, Figure 5).

Days to seedling emergence in this research were longer compared to other studies. Emergence in the field ranged from 10 days to 14 days (Table 4). Miya and Modi (2017) showed that Pongala local landraces of bambara groundnut with different colors had begun to germinate 50-60% on the second day and on the 14th day with a germination rate of 90-98%. Black Sumedang, Black Gresik, and Brown Gresik showed the earliest seedling emergence (11 days) but not significantly different from other landraces except Cream Sumedang which was the longest (14 days). This result indicated that there is no variation between landraces in days to emergence.

Table 2. Effect of cultivation method to all characters

Character	Farmer's method	CFF's method
Plant height (cm)	35.5 a	38.4 a
Number of leaves	76.8 a	83.1 a
Canopy diameter (cm)	60.0 a	60.3 a
Leaf length (mm)	94.0 a	94.3 a
Leaf width (mm)	34.6 a	32.2 a
Number of days to 50% flowering	66.4 a	68.1 a
Number of pod per plant	37.6 a	36.0 a
Seed length (mm)	13.8 a	13.7 a
Seed width (mm)	96.3 a	96.3 a
Fresh pod weight/plant (g)	110.7 a	62.7 b
Dry pod weight/plant (g)	39.3 a	37.2 a
Pod number/plant	37.6 a	36.0 a

Note: Numbers followed by the same letters in the same columns are not significantly different at the level of 5% LSD.

Table 3. Testa color, leaf shape and pod shape of bambara groundnut Indonesian landraces

Qualitative character	Cream Sumedang	Brown Sumedang	Black Sumedang	Black Gresik	Brown Gresik	Black Madura	Black Tasikmalaya	Black Sukabumi
Testa Color	80% brown, 20% cream	80% brown, 20% cream	95% black, 5% brown	90% black, 10% brown	90% black, 10% brown	90% black, 10% brown	100% black	100% black
Leaf shape	Oval	Oval	Lanceolate	Elliptic	Elliptic	Elliptic	Lanceolate	Elliptic
Pod shape*	2	2	2	3	3	3	2	2

Note: *Pod shape classification: 1. Without point, 2. Ending in a point, round on the other side, 3. Ending in a point, with nook on the other side (Figure 3)

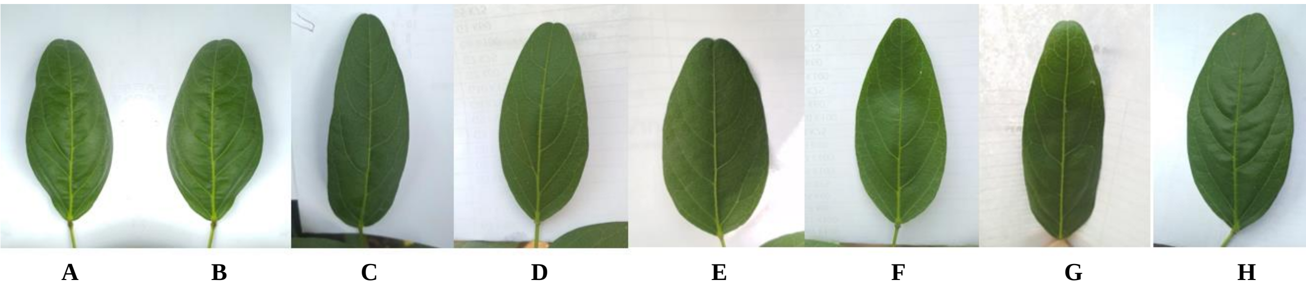

Figure 2. Leaf shape of bambara groundnut Indonesian landraces. A. Cream Sumedang, B. Brown Sumedang, C. Black Sumedang, D. Black Gresik, E. Brown Gresik, F. Black Madura, G. Black Tasikmalaya, H. Black Sukabumi

The longest terminal leaflet was observed in Black Sumedang and Black Tasikmalaya landraces, demonstrating that lanceolate leaf is longer than elliptic leaf (Black Gresik, Black Madura, and Black Tasikmalaya) except Brown Gresik but not significantly different with oval leaf (Cream Sumedang and Brown Sumedang) (Table 4). Cream Sumedang and Brown Sumedang (oval leaves) had wider leaves than elliptic (Cream Sumedang and Brown Sumedang) or lanceolate (Black Sumedang and Black Tasikmalaya). The number of leaves among the landraces was not significantly different. Brown Gresik showed the highest number of leaves (63, followed by Black Sukabumi (62).

Black Sumedang showed the widest canopy with 59.1 cm in diameter, while the smallest canopy was Black Gresik (52.5 cm). According to Ezedinma and Maneke (1985), bambara groundnut can be grouped based on the diameter of the plant canopy, namely the type of bunch (canopy diameter <40 cm), semi bunch (canopy diameter 40-80 cm), and spreading (canopy diameter> 80 cm). Therefore, based on this classification, all Indonesian landraces in this study belong to semi bunch plant group.

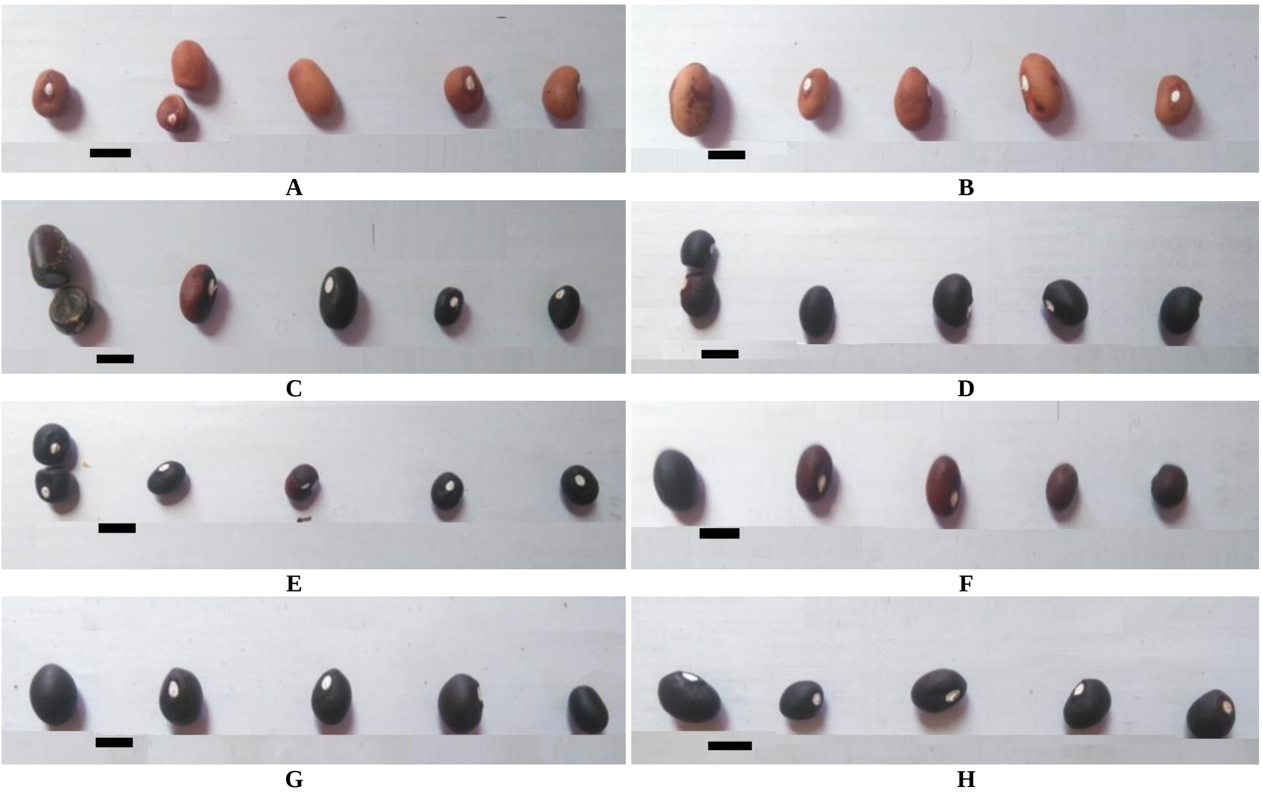

Figure 3. Testa color of bambara groundnut Indonesian landraces. A. Cream Sumedang, B. Brown Sumedang, C. Black Sumedang, D. Black Gresik, E. Brown Gresik, F. Black Madura, G. Black Tasikmalaya, H. Black Sukabumi. Bar = 1 cm

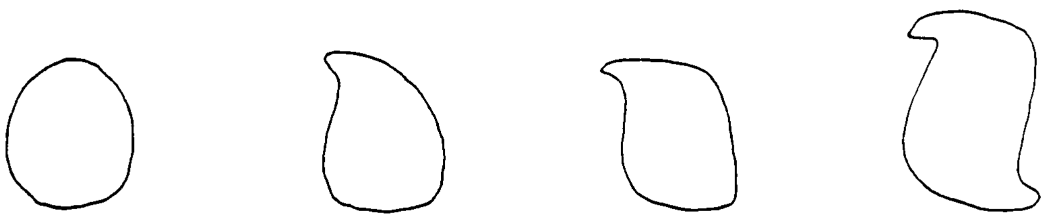

Figure 4. Pod shape classification based on bambara groundnut descriptor (IPGRI et al. 2000)

Figure 4. Pod shape of bambara groundnut Indonesian landraces. A. Cream Sumedang, B. Brown Sumedang, C. Black Sumedang, D. Black Gresik, E. Brown Gresik, F. Black Madura, G. Black Tasikmalaya, H. Black Sukabumi

Table 4. Days to emergence, plant height, leaf length, leaf width, number of leaves and canopy diameter

Landrace	Days to emergence	Plant height (cm)	Terminal leaflet length (mm)	Terminal leaflet width (mm)	Number of leaves	Canopy diameter (cm)
CRSM	14 b	36.5 ab	95.0 abc	41.1 a	49 a	56.0 ab
BRSM	13 ab	37.4 a	96.2 ab	41.3 a	47 a	58.8 ab
BLSM	11 a	36.5 ab	101.1 a	31.4 b	58 a	59.1 a
BLGR	11 a	30.5 c	85.3 cd	29.9 b	57 a	52.5 b
BRGR	11 a	33.1 bc	93.9 abc	29.4 b	63 a	55.7 ab
BLMA	12 a	30.4 c	80.9 d	30.2 b	57 a	52.6 ab
BLTA	13 ab	34.0 abc	98.6 a	28.5 b	50 a	55.5 ab
BLSK	13 ab	32.45 bc	85.8 bcd	32.7 b	62 a	55.8 ab

Note: Numbers followed by the same letters in the same columns are not significantly different at the level of 5% LSD. *CRSM: Cream Sumedang, BRSM: Brown Sumedang, BLSM: Black Sumedang, BLGR: Black Gresik, BRGR: Brown Gresik, BLMA: Black Madura, BLTA: Black Tasikmalaya, BLSK: Black Sukabumi

Table 5. Number of days to 50% flowering, number of pods per plant, seed length and seed width

Landrace	Number of days to 50% flowering	Number of pods per plant	Dry pod weight/plant	Seed length (mm)	Seed width (mm)
CRSM	61.0 ab	23.6 abc	36.0 abc	15.7 a	10.3 ab
BRSM	56.3 a	17.9 c	24.4 c	16.3 a	11.3 a
BLSM	62.2 ab	33.1 abc	36.8 abc	15.2 ab	10.3 ab
BLGR	72.2 bc	54.5 ab	44.2 abc	11.3 cd	8.5 c
BRGR	73.3 bc	55.2 a	49.4 a	12.3 c	8.3 c
BLMA	77.2 c	44.9 abc	38.9 abc	11.0 d	8.8 c
BLTA	68.0 abc	21.5 bc	28.1 bc	14.0 b	9.3 bc
BLSK	67.8 abc	43.8 abc	48.1 ab	14.0 b	10.2 ab

Note: Numbers followed by the same letters in the same columns are not significantly different at the level of 5% LSD

Table 5 showed that all landraces from Sumedang were early in flowering (56-62 days), and all landraces from Gresik and Madura were late in flowering (72-73 days). Meanwhile, Black Sukabumi and Black Tasikmalaya (68 days) were classified as average days in flowering because they were not significantly different from Sumedang and Madura. The slow day of germination (10.5 - 14.2 days) (Table 4) may be contributed to the late flowering in this study. According to Mkandawire (2007), flowering of bambara groundnut starts 30 to 35 days after sowing and may continue until the end of the plant's life. The result of Berchie et al. (2010) showed that bambara groundnut Burkina, Zebra, and Mottled cream landraces flowered 50% in ± 40 days after planting. Meanwhile, Manggung et al. (2016) reported that Bambara groundnut Black Sumedang, Brown Sumedang, Brown Bogor, and Black Sukabumi landraces reached 50% flowering in ± 45 days after planting.

Brown Gresik landrace had the highest number of pods (55.2) per plant, followed by Black Gresik (54.5), Black Sukabumi (43.8), Black Sumedang (33.1) and Cream Sumedang (23.6) which were not significantly different. Meanwhile, Brown Sumedang had the lowest number of pods (17.9). Rainfall was quite high during the harvest period (273 mm in December). High number of pods in Gresik landraces showed that they were quite suitable for cultivation in August to December compared to the other landraces. Dry pod weight per plant showed an almost similar result with the number of pods per plant. Brown Gresik landrace had the highest dry pod weight per plant and showed the significantly difference between Black Tasikmalaya and Cream Sumedang.

The seed length data showed that Brown Sumedang was the biggest seed and not significantly different from Cream Sumedang and Black Sumedang, while Black Madura was the smallest and not significantly different from Black Gresik (Table 5). Seed width also showed almost similar results with seed length. All Sumedang landraces tend to produce seed with the big size while all Gresik and Madura produced the small seed. All landraces tend to have the same classification on seed size character as number of days to 50% flowering. This indicates a relationship between seed size and flowering time. Landraces with large size seeds tend to flower faster than the ones with small seeds.

Cluster analysis

Cluster analysis was made based on qualitative and quantitative morphological markers. The results of data analysis showed similarity among characters in eight landraces in the form of dendrogram. Morphological distances between landraces ranged from 66.44% to 94.33%. This is almost similar to the result of Siise and Massawe (2012) that showed distances among Ghanaian landraces of bambara groundnut ranged from 70% to 92%. It can be seen that the smaller the similarity values, the greater the differences among these individuals. There were two main clusters with 88.28% similarity. High value of similarity means that Indonesian landraces of bambara groundnut have narrow diversity. In its development, narrow diversity of Indonesian landraces requires plant crossing to create new variations of genetic.

The first cluster consisted of Brown Sumedang, Black Tasikmalaya, and Black Sumedang with 89.64% similarity (Figure 6). The similar character of this cluster was wide leaf area, early flowering, and big size of seed (Table 6). This is not surprising because both landraces originated from nearby areas, Sumedang and Tasikmalaya are in Province of West Java, Indonesia. If the similarity level is added, this cluster can be divided into two subclusters with Black Sumedang separated from this cluster. If the similarity is added more, Black Tasikmalaya will separate from this cluster. The difference between Black Sumedang and Black Tasikmalaya with this cluster is in leaf shape and testa color. Black Sumedang and Tasikmalaya are lanceolate in leaf shape while Cream and Brown Sumedang are oval. In testa color, Cream and Brown Sumedang are dominated by brown testa, while Black Tasikmalaya and Black Sumedang are dominated by black testa.

The second cluster consisted of four landraces (Black Sukabumi, Brown Gresik, Black Madura, and Black Gresik) with 92.51% similarity. The similar characters of this cluster were elliptic leaf shape, having more number of leaves and pods, and late to flowering (Table 6). This cluster has a character to potentially produce more pods. This cluster can be divided into two subclusters with Black Sukabumi separated from East Java landraces (Brown Gresik, Black Madura, and Black Gresik). The most similar landraces in this research were Black Gresik and Brown Gresik with 94.33% similarity (Figure 6). This is not surprising because both landraces originated from proximity areas.

In order to develop a variety, prospective parental lines must have a great degree of diversity (Sanjaya et al. 2002). Cream Sumedang or Brown Sumedang from the first cluster and Black Gresik or Brown Gresik from the second cluster have the farthest distances for developing improved variety of bambara groundnut. Cream and Brown Sumedang have superior traits in seed size, brown/cream testa color and early flowering. According to Hudu and Saaka (2011), cream testa is preferred in the market and farmers in Ghana. While Black Gresik and Brown Gresik are small size in seed, later in flowering but better than Cream and Brown Sumedang in number of pods and dry pod weight/plant.

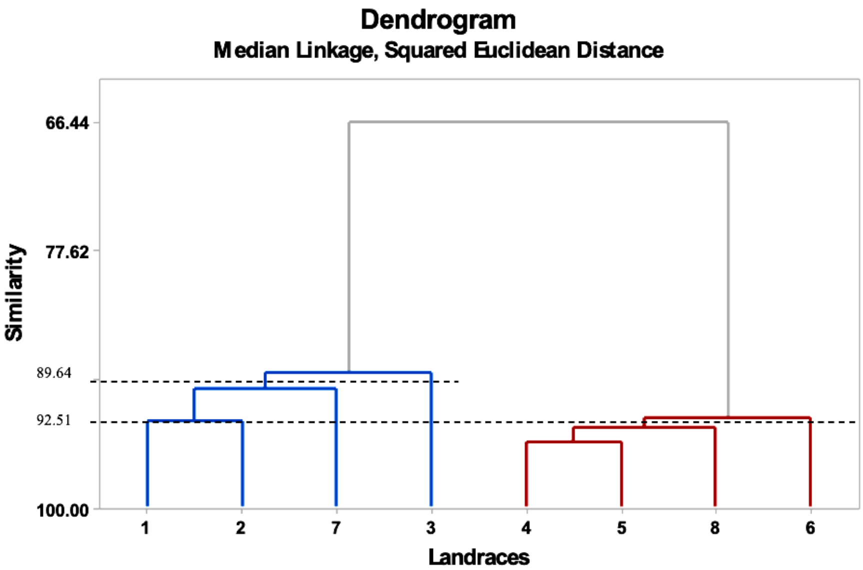

Figure 5. Dendrogram of eight Indonesian landraces of bambara groundnut based on morphological characters. 1. Cream Sumedang, 2. Brown Sumedang, 3. Black Sumedang, 4. Black Gresik, 5. Brown Gresik, 6. Black Madura, 7. Black Tasikmalaya, 8. Black Sukabumi

Table 6. Similar characters in each cluster

Cluster	Landraces	Similar character
1	Cream Sumedang, Brown Sumedang, Black Tasikmalaya, Black Sumedang	High plant height, wider leaf area, early flowering (62 days), big size seed (15x10 mm),
2	Black Sukabumi, Brown Gresik, Black Madura, Black Gresik	More number of leaves, late to flowering (73 days), more number of pods, elliptic leaf shape.

Table 7. Triplicate allelic scoring of Indonesian bambara groundnut landraces using Primer D11

Replication	Cream Sumedang	Brown Sumedang	Black Sumedang	Black Gresik	Brown Gresik	Black Madura	Black Tasikmalaya	Black Sukabumi
1	195/195	195/195	195/195	197/197	195/195	197/197	195/195	195/195
2	195/195	195/195	195/195	197/197	195/195	195/195	195/195	195/195
3	195/195	195/195	195/195	197/197	195/195	197/197	195/195	195/195

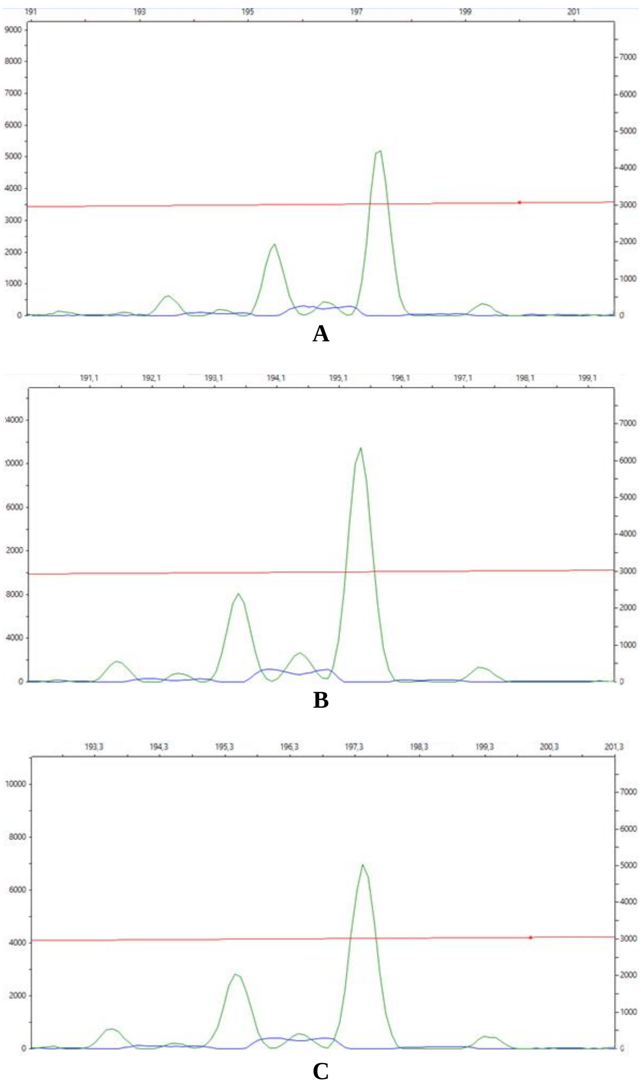

Figure 6. Length of PCR product using capillary electrophoresis scored by Peak Scanner at primer D-11. A. Black Gresik, B. Cream Sumedang, C. Black Madura

SSR marker using capillary electrophoresis

Amplification using three selected pairs of primers was successfully conducted. Polymorphic band was not observed using Primer 33 (172/172 bp) and Primer 37 (256/256 bp) in all samples. Polymorphism was found using Primer D11 in the tested genotypes (Table 7). The length fragment difference between Black Gresik and Black Madura with other landraces showed that D-11 primer can differentiate Black Gresik and Black Madura landrace from other landraces. Interestingly, although collected from the same origin, SSR Primer D11 could distinguish between Black Gresik landrace and Brown Gresik landrace. The different amplification score between 3 replications of Black Madura has suggested that Black Madura was not pure line. There is a possibility of mixing Madura landrace seeds with other landraces from the farmers.

Although SSR can also be used to check heterozygosity (Ho et al. 2016), in our study, however, none of the landraces showed as heterozygous genotypes. It is known that bambara groundnut is a self-pollinating plant (Heller et al. 1997). Obtaining pure inbreed line is important for breeding work of bambara groundnut. Homozygous parental lines having preferred traits need to be identified first prior development of superior bambara groundnut. The use of sufficient number of SSP, therefore, could help better in identifying pure inbred lines.

In conclusion, there were two main clusters of Indonesian landraces of bambara groundnut with 88.28% similarity. The first cluster was Cream, Brown Sumedang, Black Sumedang, and Black Tasikmalaya, and the second cluster was Black Sukabumi, Brown Gresik, Black Madura, and Black Gresik. The result based on SSR marker with capillary electrophoresis indicated Black Gresik and Black Madura landraces were different from other Indonesian landraces. Cream Sumedang or Brown Sumedang from the first cluster and Black Gresik or Brown Gresik from the second cluster have the farthest distances for developing improved variety of bambara groundnut.

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