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Morphological variability and cluster analysis of 16 bambara groundnut (*Vigna subterranea*) genotypes

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Abstract. *Hamdi MFFA, Ilyas S, Qadir A, Mayes S. 2024. Morphological variability and cluster analysis of 16 bambara groundnut (*Vigna subterranea*) genotypes. Biodiversitas 25: 97-106.* Identifying morphological and germination characters in bambara groundnut (*Vigna subterranea* (L.) Verdc.) is important to determine the advantages and disadvantages of several genotypes. This study aimed to identify the characteristics of 16 bambara groundnut genotypes based on morphological markers, NDVI score, and germination variables. This study was conducted in Sumedang, West Java, Indonesia in March-July 2018. The experiment was arranged in a randomized complete block design with one factor: genotype, which consisted of 16 genotypes originating from Indonesia and Africa. The NDVI score did not affect the yield produced. Tiga Nicuru, DodR-R II, M-14 Gresik, Black Sukabumi, and Black Madura were the genotypes with the highest germination speed, while LunT was the lowest. Cluster analysis showed that bambara groundnut genotypes are classified into 4 clusters. The first cluster belongs to Sumedang and Sukabumi, the second belongs to Gresik, Madura, and Tasikmalaya, the third comes from West Africa (LunT and Tiga Nicuru), and the fourth comes from East Africa (IITA 686 and DodR-R II) and Southern Africa (S 19-3, Uniswa R and Uniswa R/G). The low similarity (28%) between genotypes from Indonesia and Africa shows that there are many differences in morphological characteristics. This high diversity is beneficial for creating superior cultivars.

Keywords: Bambara groundnut, genotypes, germination speed, morphological marker, NDVI, *Vigna subterranea*

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is one of the grain legume crops indigenous to Africa (Majola et al. 2021). It is an alternative food source producing protein and carbohydrates (Rahmah et al. 2020). In dried seeds, bambara groundnut contains 64.4% carbohydrate, 23.6% protein, 6.5% fat, and 5.5% fiber and is rich in minerals (Halimi et al. 2019). The uses of bambara groundnut in improving food systems is hindered by a lack of agricultural policy around the value chain, consistent phenological development, i.e., sensitivity to long photoperiods and a phenomenon referred to as hard-to-cook during post-storage processing (Mateva et al. 2023). In Indonesia, bambara groundnut is known as Bogor peanuts, but in some regions, they have different names.

Bambara groundnut has the potential to replace soybean as an important legume in Indonesia, because it is easily grown and drought tolerant (Alhamdi et al. 2020). According to Feldman et al. (2019), bambara groundnut is most impressive in drought tolerance, encompassing all three composite traits of drought escape, avoidance, and tolerance. Moreover, Mayes et al. (2019) stated that bambara groundnut can be used to contribute more to climate change-ready agriculture. Farmers in several areas in Indonesia plant side crops, such as peanuts (*Arachis hypogaea* L.) or other legumes that can grow during the dry season, one of the side crops is bambara groundnut.

Madura, Gresik (East Java), Sukabumi, Sumedang, and Tasikmalaya (West Java) are some areas where farmers cultivate bambara groundnut as a side crop during the dry season. The areas mentioned have native plants or landraces cultivated yearly (Suryati 2019). Some landraces have different seed coat colors, which are suspected of having differences. The genetic diversity of bambara groundnut in Indonesia has been described in several studies. Sobari and Wicaksana (2017) stated that some bambara groundnut landraces in West Java showed a broad variation in fresh pod weight, dry pod weight, the weight of 100 seeds, and weight per plot character. Fatimah et al. (2018) showed that the similarity among 12 Madurese bambara groundnut is 52% based on morphological markers and 51% based on RAPD markers. According to Alhamdi et al. (2020), the similarity of eight landraces of Indonesian bambara groundnut is 66.4% based on morphological markers.

The genetic diversity of bambara groundnut in Africa has been described in several studies. The findings from several studies indicate that bambara groundnut diversity is broad enough based on morphological characters (Gbaguidi et al. 2018) and molecular characters (Molosiwa et al. 2015). Khan et al. (2021) stated that most of the study findings were highly country-specific, involving germplasm from Southeast Asia and Africa's major agro-climatic zones. Based on this research, it is important to know the superior and non-superior characteristics of several genotypes based on their origin.

In addition to character identification, Normalized Difference Vegetation Index (NDVI) measurements can be carried out to find out whether there is a correlation between the characteristics of the bambara groundnut and the NDVI value. NDVI measurements are used to measure N levels in various plants such as corn (Edalat et al. 2019), wheat (Aranguren et al. 2020), and rice (Jiang et al. 2021). If there is a significant correlation between the NDVI value and one of the traits, this fairly easy NDVI measurement can be used as an alternative in doing observations. Germination is one of the important stages in legume cultivation. Several studies indicate a correlation between legume seed coat color and seed germination, which is important to study. This study aimed to identify the characteristics of 16 bambara groundnut landraces based on morphological markers, NDVI score, and germination variables.

MATERIALS AND METHODS

Research sites

The field experiment was conducted in Mekarmulya Village, Situraja District, Sumedang Regency, West Java, Indonesia (6°51'09.4" S and 108°01'01.5" E) from March to July 2018. Before the experiment began, soil samples were taken and analyzed at the Testing Laboratory of the Department of Soil Science and Land Resources, IPB University, Bogor, Indonesia.

Plant materials

The plant materials used in this research were bambara groundnut seeds of 16 genotypes listed in Table 1. The first 8 genotypes were landraces from Indonesia, with seed coat colors according to their names. In comparison, the 8 genotypes after that were core lines of collections from Crops for the Future (CFF), Semenyih, Malaysia.

Procedures

Cultivation

Land preparation began with clearing weeds, loosening the soil, and applying chicken manure. The manure was made by mixing dry chicken manure and rice straw in a ratio of 2:1 and then burning it to ashes. The dose of manure used in this experiment was 2 tons ha^{-1}, done 2 weeks before planting. The application of fertilizers [0.3 g urea (45% N), 1 g SP-36 (36% P_2O_5), 1 g KCl (60% K_2O)] were on each plant hole at 5 weeks after planting (WAP). Earthing was done twice during flowering (35-38 days after planting) and pod formation (14 days after flowering). Weeding was done 3 WAP and 6 WAP. Planting was done when the rainfall was high enough (Table 2), so watering was not given. According to the Department of Agriculture, Forestry and Fisheries of South Africa (2016), the rainfall that can be tolerated is 500-1,200 mm during the growing season. Therefore, this study was carried out under ideal conditions with a total rainfall of 454.7 mm (Table 2).

Table 1. Rainfall at Situraja district, Sumedang, West Java, Indonesia during the planting period

Month	Rainfall (mm)	Rainy days
March*	102.0	7
April	256.5	16
May	58.0	8
June	38.2	5
July	0	0
Total	454.7	36

Notes: *starting on 24 March 2018 (Rainfall data was taken from an ombrometer managed by the Cisitu District Regional Technical Implementation Unit (UPTD), Sumedang, West Java, Indonesia 2018)

Table 2. List of bambara groundnut genotypes used in the study, showing their origin and seed coat color

Genotype	Source	Country of origin
Cream Sumedang	Local farmers	Indonesia
Brown Sumedang	Local farmers	Indonesia
Black Sumedang	Local farmers	Indonesia
Black Gresik	Institut Pertanian Bogor, Bogor, Indonesia	Indonesia
Brown Gresik	Institut Pertanian Bogor, Bogor, Indonesia	Indonesia
Black Madura	Universitas Brawijaya, Malang, Indonesia	Indonesia
Black Tasikmalaya	Institut Pertanian Bogor, Bogor, Indonesia	Indonesia
Black Sukabumi	Institut Pertanian Bogor, Bogor, Indonesia	Indonesia
M-14 Gresik	Crops for the future	Indonesia
LunT	Crops for the future	Sierra Leone
Tiga Nicuru	Crops for the future	Mali
IITA 686	Crops for the future	Tanzania
DodR-R II	Crops for the future	Tanzania
S 19-3	Crops for the future	Namibia
Uniswa R	Crops for the future	Swaziland
Uniswa R/G	Crops for the future	Swaziland

Research design

The experiment was arranged in a randomized completely block design with one factor, namely genotype. The genotype factor consists of 16 genotypes (Table 1) and each factor consists of 3 repetitions (block). The experiment was carried out on an area of 21 × 8 m consisting of three blocks of 7 × 8 m each. Each block contained 16 plots with genotype treatment. The spacing used was 25 × 50 cm, so there were 21 plant populations in each plot.

Morphological characters observation

Morphological characters consisted of quantitative characters, such as plant height, number of trifoliate leaves, canopy diameter, days to 50% flowering, harvest age, fresh pod weight per plant, dry pod weight per plant, number of pods per plant, pod length, pod width, seed length, seed width and speed of germination; and qualitative characters, such as leaf shape and seed coat color. Plant height, the number of trifoliate leaves, and canopy diameter were measured at 10 WAP. Dry pod weight per plant was weighed after being sun-dried for 7 × 12 hours. Speed of germination is one indicator to assess the quality of bambara groundnut seeds (Fitriesa et al. 2016). The germination speed was calculated from the results of the seed germination test in sterile sand media. The seeds used in the germination test were the results of this study after being sun-dried and stored for 3 months. This is because bambara groundnut seed has been known to have a dormant character, especially in the early period after harvesting (Sari et al. 2021). Based on ISTA (2018), the germination of bambara groundnut was observed on the 5th (first count) and 10th day after planting (final count). However, for bambara groundnut seeds from the West Java landraces, the germination test is appropriate on the 7th and 14th days after planting (DAP) (Ilyas and Sopian 2013).

Normalized Difference Vegetation Index (NDVI) measurements were conducted from 3 WAP to 10 WAP using a color spectrum sensor from the Trimble GreenSeeker tool. The GreenSeeker was used to measure the plant canopy's spectral reflectance at different wavelengths. GreenSeeker used lights of two wavelengths (774 nm and 656 nm), so that the measurements could be used to form the NDVI (Barker et al. 2016). This tool has been used to estimate yields of ryegrass plants (Wang et al. 2019), visual estimates of defoliation by late leaf spot of peanut (*A. hypogaea*) and can be used in automated phenotyping of genetic populations to map the QTL controlling resistance to stripe rust (Pretorius et al. 2016).

Data analysis

Morphological characters were scored and analyzed using ANOVA performed by Statistical Tool for Agricultural Research (STAR). 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 NTSYSpc.v2.10e (cluster analysis). Each data was then tested for Pearson correlation using STAR software.

RESULTS AND DISCUSSION

Morphological characters

ANOVA results showed significant differences in all quantitative morphological characters (Table 3). Variables of plant height and canopy diameter showed a tendency for landraces from Indonesia to be taller in plant height and wider in canopy diameter than several genotypes from Africa, except Uniswa R whose canopy diameter was not significantly different from Cream/Brown Sumedang. Tiga Nicuru is the shortest genotype, with an average height of 24.33 cm (Table 3). The number of M-14 Gresik leaves (123.3) was significantly higher than the other genotypes (Table 3). Only 3 plants were growing in each replication plot of the M-14 Gresik genotype. This made M-14 Gresik better in vegetative growth than the others due to tighter nutrient competition.

Table 3. Plant height, number of trifoliate leaves, canopy diameter, days to 50% flowering, and leaf shape

Genotype	Plant height (cm)	Number of trifoliate leaves	Canopy diameter (cm)	Days to 50% flowering (DAP)	Leaf shape
Cream Sumedang	36.67 a	54.67 cd	60.00 a	37.33 cde	Elliptic
Brown Sumedang	36.67 a	57.33 bcd	58.00 abc	37.67 de	Elliptic
Black Sumedang	32.67 bcde	63.67 bcd	61.67 a	36.33 abcd	Lanceolate
Black Gresik	33.67 abcd	79.67 bc	58.00 abc	38.00 de	Elliptic
Brown Gresik	32.00 def	82.33 b	61.33 a	37.00 bcde	Elliptic
Black Madura	35.67 abc	79.33 bc	59.00 ab	37.00 bcde	Lanceolate
Black Tasikmalaya	36.00 ab	74.67 bc	56.67 abcd	37.33 cde	Lanceolate
Black Sukabumi	36.00 ab	69.00 bcd	60.00 a	37.00 bcde	Elliptic
M-14 Gresik	32.67 bcde	123.33 a	63.33 a	39.33 e	Lanceolate
LunT	28.00 g	45.33 d	41.33 ef	36.33 abcd	Lanceolate
Tiga Nicuru	24.33 h	66.67 bcd	35.67 f	35.67 abcd	Lanceolate
IITA 686	31.33 defg	58.67 bcd	47.33 cde	33.67 a	Elliptic
DodR-R II	29.67 efg	67.00 bcd	46.67 def	34.33 ab	Elliptic
S 19-3	29.00 fg	56.33 bcd	48.67 bcde	34.67 abc	Oval
Uniswa R	32.33 cdef	71.33 bcd	56.67 abcd	35.33 abcd	Elliptic
Uniswa R/G	31.67 def	60.67 bcd	47.00 cde	38.00 de	Elliptic

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

Days to 50% flowering were relatively more uniform, about 33-38 days after planting (DAP) (Table 3). This was suspected by the start of the dry season in April, marked by a decrease in rainy days; this was exacerbated by not doing watering at all. The fastest flowering genotype was IITA 686, which could flower at 33.67 DAP; that was not significantly different from Black Sumedang, LunT, Tiga Nicuru, DodR-R II, S 19-3, and Uniswa R genotypes. Meanwhile, the slowest flowering was M-14 Gresik genotype (39.33 DAP) and was not significantly different from Cream Sumedang, Brown Sumedang, Black Greesik, Brown Gresik, Black Madura, Black Tasikmalaya, Black Sukabumi and Uniswa R/G genotypes. The leaf shapes were quite diverse, with the discovery of 9 genotypes with elliptical leaves, 6 with lanceolate leaves, and 1 with oval leaves (Table 3). The leaf shape was observed by looking at the shape of most leaves in each genotype at 10 WAP. Sometimes, there was a slight change in the leaf shape of the genotype in the first week until harvest, for example in Cream and Brown Sumedang genotypes, some leaves were shaped like ovals at the beginning of growth but shed and grew elliptical leaves at 10 WAP.

Some genotypes showed high results on fresh pod weight, i.e., DodR R-II, all Sumedang genotypes, Sukabumi, LunT, and Uniswa R genotypes (Table 4). However, regarding dry pod weight, Cream Sumedang was not included in the highest; IITA 686 was among the highest and was not significantly different from Black Sumedang (44.83 g), Uniswa R (41.20 g) and DodR-R II (40.90 g) (Table 4). The highest number of pods was dominated by African genotypes, i.e., IITA 686, DodR-R II, S 19-3, Uniswa R, and Uniswa R/G, but not significantly different from Black Sumedang, Black Gresik, Black Madura and Sukabumi. The five genotypes from Africa produced ±38 pods, while the four genotypes from Indonesia only produced ±29 pods per plant (Table 4).

Tiga Nicuru genotype has the fastest harvest times (99.33 DAP) and was significantly different from the rest (Table 4). Therefore, although the flowering time was almost the same for all genotypes (Table 3), the formation and filling of pods in the Tiga Nicuru genotype was faster; hence, the harvest time was faster. Meanwhile, several genotypes classified as long-harvesting were Uniswa R, Uniswa R/G, IITA 686, M-14 Gresik, Black Tasikmalaya, and Black Sumedang. Harvest age in this study was determined by leaf dryness; harvesting was done if 50% of the plant population had dry leaves.

A black seed coat color dominated bambara groundnut from Indonesia and only the genotype from Sumedang had a brown color. Meanwhile, the genotype from Africa had three types of seed coat color, i.e., black, beige, and brownish red (Table 4).

Pod length ranged from 13.17 to 23.67 mm (Table 5). The longest pod belonged to Brown Sumedang genotype (23.67 mm), which was not significantly different from the other Sumedang genotypes and Black Sukabumi genotype. The shortest pod was shown by the S 19-3 genotype (13.17 mm), which was not significantly different from IITA 686, Black Madura, Black Gresik, M-14 Gresik, and Uniswa R/G genotypes. The widest pod belonged to Brown Sumedang genotype (18.67 mm), significantly different from the other genotypes.

Black Tasikmalaya, Black Sukabumi, Black Gresik, Cream, and Brown Sumedang genotypes had the longest seeds ±14 mm. The 5 genotypes differed significantly higher than all genotypes from the CFF collection. Tiga Nicuru (8 mm) and S 19-3 genotypes (8.33 mm) were the shortest seeds. In addition, seed width tended to be more uniform across all genotypes. Black Tasikmalaya and Sukabumi genotypes were the widest seeds (11.67 mm), significantly different from the other genotypes.

The length of the pods and seeds was measured after drying in the sun for 7 × 12 hours to decrease the seed size. Based on the pod size and seed size, there were differences in the degree of shrinkage between one genotype and another. For example, Brown Sumedang genotype, which had the largest pods, had smaller seed sizes than the Black Tasikmalaya genotype, which had smaller pods than Brown Sumedang genotype. This phenomenon requires further research.

Table 4. Fresh pod weight, dry pod weight and number of pods per plant, harvest age, and seed coat color

Genotype	Fresh pod weight/ plant (g)	Dry pod weight/ plant (g)	Number of pods/ plant	Harvest age (DAP)	Seed coat color
Cream Sumedang	56.40 abc	25.30 cdef	19.27 d	112.00 ef	Brown
Brown Sumedang	66.60 abc	29.47 abcdef	20.87 d	108.67 bcde	Brown
Black Sumedang	92.60 ab	44.83 a	32.60 abcd	115.67 fg	Black
Black Gresik	45.50 c	21.53 ef	28.40 abcd	107.33 bcd	Black
Brown Gresik	44.50 c	20.97 ef	21.67 d	110.67 de	Black
Black Madura	52.73 bc	22.87 def	26.00 abcd	105.00 b	Black
Black Tasikmalaya	31.90 c	20.67 ef	19.00 d	116.67 g	Black
Black Sukabumi	88.30 ab	37.80 abcd	31.50 abcd	105.00 b	Black
M-14 Gresik	32.37 c	19.17 ef	23.43 cd	116.67 g	Black
LunT	57.13 abc	29.67 abcdef	25.50 bcd	108.67 bcde	Beige
Tiga Nicuru	37.47 c	18.00 f	20.17 d	99.33 a	Beige
IITA 686	41.10 c	29.87 abcdef	40.40 ab	116.67 g	Black
DodR-R II	96.17 a	40.90 abc	40.30 ab	106.00 bc	Brownish red
S 19-3	41.60 c	28.60 bcdef	37.80 abc	109.67 cde	Black
Uniswa R	63.33 abc	41.20 ab	32.87 abcd	119.00 g	Brownish red
Uniswa R/G	53.13 bc	34.07 abcde	41.13 a	116.67 g	Brownish red

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

Normalized Difference Vegetation Index (NDVI)

NDVI measurement results showed that the Black Madura genotype was always on top every week, with the highest peak in the 9th week (Figure 1). Meanwhile, the genotype lines of Tiga Nicuru, IITA 686, and LunT tended to be at the bottom (Figure 1). Tiga Nicuru genotype had the lowest NDVI value because it had a narrow canopy and few leaves (Table 5). The canopy diameter of genotypes from Indonesia was significantly wider than genotypes from Africa, except Uniswa R (Table 3); this was directly proportional to the NDVI score obtained. The NDVI scores for 9 WAP showed that only Tiga Nicuru, LunT, and IITA 686 genotypes were significantly lower than the other genotypes (Table 5).

Black Madura, Black Sukabumi, Brown Gresik, and Black Tasikmalaya genotypes have high NDVI scores at 7, 8, and 9 WAP (Figure 1). Of the 4 genotypes, Black Sukabumi is classified as high yield (88.30 g fresh pod weight/plant, 37.80 g dry weight pod/plant, and 31.5 number of pod/plant), while Black Madura is only high in number of pod/plant (26 pods), the rest are not classified as high yield (Table 4). This showed that Black Sukabumi genotype is quite effective in distributing nutrients to fill the pods. Meanwhile, genotypes that are classified as high yield include DodR-R II (96.17 g fresh pod weight/plant, 40.90 g dry weight pod/plant, and 40.3 number of pods/plant) and Black Sumedang (92./60 g fresh pod weight/ plant, 44.83 g dry weight pod/plant and 32.6 number of pods/plant) is not very good in NDVI score (Figure 1).

Germination

DodR-R II and Tiga Nicuru genotypes were the highest (90%) at 7 DAP germination percentage, followed by M-14 Gresik (86%), IITA 686 (84%), and Uniswa R (84%) (Table 6). The genotypes from Sumedang had the lowest percentage (0% and 12%) but higher germination at 14 DAP (98%). Tiga Nicuru, DodR-R II, and M-14 Gresik were the genotypes with the highest germination speed, while LunT was the lowest.

Table 5. Pod length, pod width, seed length, seed width, and speed of germination

Genotype	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	NDVI score at 9 WAP
Cream Sumedang	21.67 ab	15.67 b	14.33 ab	8.33 bcd	0.78 abc
Brown Sumedang	23.67 a	18.67 a	14.33 ab	8.67 bcd	0.77 abc
Black Sumedang	21.00 abc	15.00 bc	12.67 bcde	7.33 d	0.75 abc
Black Gresik	15.67 efg	12.67 defg	13.67 abc	9.33 b	0.70 abcd
Brown Gresik	20.00 bcd	15.00 bc	12.00 cde	8.67 bcd	0.83 a
Black Madura	16.00 efg	13.33 cdef	13.33 bcd	9.33 b	0.85 a
Black Tasikmalaya	19.00 bcde	14.67 bcd	15.67 a	11.67 a	0.82 ab
Black Sukabumi	21.00 abc	16.00 b	14.67 ab	11.67 a	0.83 a
M-14 Gresik	15.00 fg	10.67 g	11.33 de	8.33 bcd	0.69 abcd
LunT	18.50 bcde	13.17 cdef	12.00 cde	8.00 bcd	0.62 bcd
Tiga Nicuru	17.33 def	12.33 efg	8.00 f	7.33 d	0.53 d
IITA 686	16.50 efg	12.00 efg	10.67 e	8.67 bcd	0.59 cd
DodR-R II	18.50 bcde	14.17 bcde	11.00 e	9.00 bc	0.74 abcd
S 19-3	13.17 g	11.67 fg	8.33 f	7.67 cd	0.78 abc
Uniswa R	17.67 cdef	14.00 bcde	11.33 de	8.67 bcd	0.67 abcd
Uniswa R/G	16.33 efg	12.67 defg	11.67 cde	8.67 bcd	0.76 abc

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

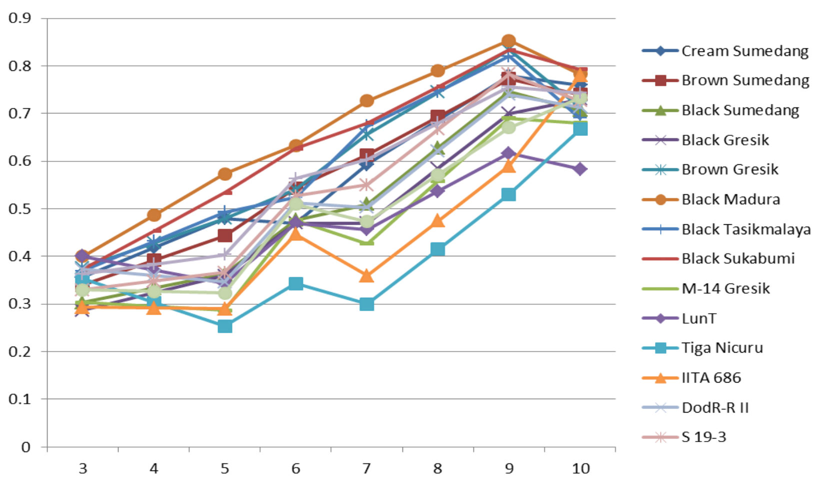

Figure 1. NDVI scores of 16 bambara groundnut genotypes

Cluster analysis

The cluster analysis results showed very large differences between the genotypes from Indonesia and Africa. The similarity between the two clusters was only 0.28 or 28% (Figure 2). Vegetative growth characters, yield, pod and seed size, and seed coat color showed different tendencies between the Indonesian and African genotype clusters. Genotypes from Indonesia tended to have larger plant and seed sizes than genotypes from Africa. However, the genotypes from Africa tended to have higher yields, and the color of the seed coats tended to be lighter than those from Indonesia.

We divided these 16 genotypes into four clusters limited to a similarity greater than 37% (Figure 2). The first cluster consists of Cream Sumedang, Brown Sumedang, Black Sumedang, and Black Sukabumi genotypes. All genotypes in this cluster come from West Java. The characteristics of the first cluster are tall plants, a wide canopy, and relatively large pods and seeds. The second cluster comprises Black Gresik, Black Madura, Brown Gresik, Black Tasikmalaya, and M-14 Gresik genotypes. All genotypes in the second cluster came from East Java except Black Tasikmalaya. The characteristics of this cluster are the large number of leaves and low yields (pod weight and number of pods). The third cluster consists of LunT and Tiga Nicuru genotypes. Both are from West Africa (Mali and Sierra Leone). The characteristics of the third cluster are the plants are short, the canopy is narrow, the number of leaves is small, the harvest time is relatively fast, and the seed coat color is beige. The fourth cluster comprises IITA 686, S19-3, Uniswa R, Uniswa R/G, and DodR-RII. IITA 686 and DodR-RII genotypes were from Tanzania (East Africa), the S 19-3 genotype was from Namibia (Southern Africa), while Uniswa R and Uniswa R/G genotypes were from Swaziland (Southern Africa). The characteristic of the fourth cluster was the high number of pods per plant. The germination speed had a significant positive correlation with the number of leaves and a significantly negative correlation with pod length and seed length (Table 7). There is an indication that the longer the seed size, the slower it germinates. The correlation between the number of leaves and the germination speed is quite difficult to explain what causes it, but this can be used as a characteristic that plants with many leaves and small seed sizes like Tiga Nicuru (Figure 3) tend to germinate quickly.

Correlation among character

The results of Pearson correlation showed that several variables are positively correlated (Table 7). Pod width, seed length and width had a highly significant positive correlation with plant height, while pod length had a significant positive correlation (Table 7). This indicates a close relationship between pod and seed size with plant height; the larger the pod and seed size, the taller the plant. Likewise, canopy diameter was significantly positively correlated with pod and seed size, except with seed width. This reinforces the assumption that the bigger the seed, the taller the plant and the wider the canopy. Tall plant and wide canopy are characteristics of the bambara groundnut from Indonesia (Table 3), especially the Black Sukabumi, Black Tasikmalaya, Cream Sumedang, and Brown Sumedang genotypes, which also have larger pod and seed sizes than the other genotypes (Table 5).

Table 6. Germination percentage and germination speed of 16 bambara groundnut genotypes

Genotype	Germination percentage		Germination speed (%/etmal)
	7 DAP	14 DAP	
Cream Sumedang	0 f	98 ab	17.0 gh
Brown Sumedang	0 f	98 ab	16.2 h
Black Sumedang	12 def	98 ab	18.7 fgh
Black Gresik	34 cd	98 ab	20.4 defg
Brown Gresik	56 bc	98 ab	23.5 bcde
Black Madura	78 ab	100 a	25.1 abc
Black Tasikmalaya	26 de	100 a	22.0 cdef
Black Sukabumi	70 ab	100 a	24.4 abc
M-14 Gresik	86 a	100 a	26.1 ab
LunT	4 ef	70 d	11.8 i
Tiga Nicuru	90 a	100 a	27.8 a
IITA 686	84 a	90 bc	23.9 bcd
DodR-R II	90 a	96 abc	27.8 a
S 19-3	68 ab	96 abc	22.9 bcde
Uniswa R	84 a	94 abc	25.2 abc
Uniswa R/G	36 cd	88 c	20.1 efg

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

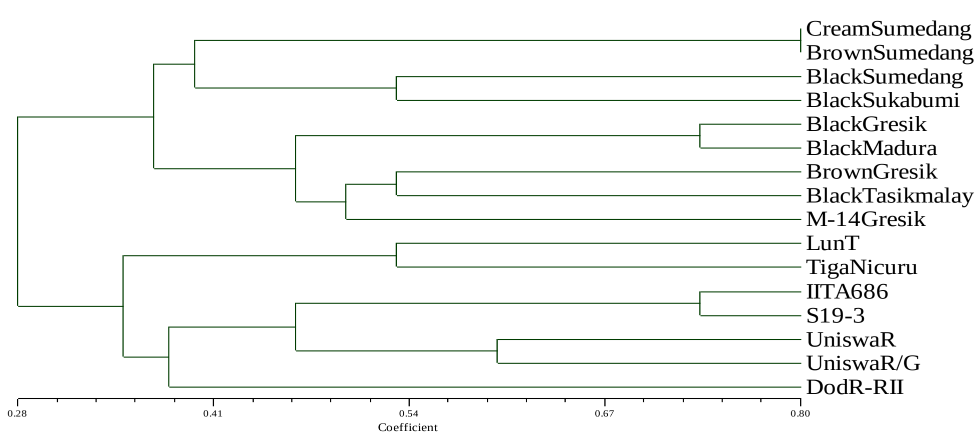

Figure 2. Cluster analysis of 16 genotypes of bambara groundnut

Figure 3. Bambara groundnut plants 10 WAP and dry seeds. A. Cluster 1: Brown Sumedang; B. Cluster 2: Black Madura; C. Cluster 3: Tiga Nicuru; D. Cluster 4: Uniswa R/G

Table 7. Correlation between morphological characters

Traits	FPW	DPW	NOP	50DF	HA	PH	NTL	CD	PL	PW	SL	SW	SG
DPW	0.875**												
NOP	0.616**	0.775**											
50DF	-0.115ns	-0.207ns	-0.284ns										
HA	-0.149ns	-0.099ns	-0.131ns	0.038ns									
PH	0.049ns	-0.019ns	-0.188ns	0.354*	0.040ns								
NTL	-0.193ns	-0.281ns	-0.188ns	0.333*	-0.166ns	0.180							
CD	0.086ns	-0.043ns	-0.195ns	0.261ns	-0.150ns	0.659**	0.580**						
PL	0.408**	0.275ns	-0.197ns	0.093ns	0.126ns	0.326*	-0.191ns	0.292*					
PW	0.401**	0.281ns	-0.118ns	0.071ns	0.094ns	0.497**	-0.199ns	0.316*	0.847**				
SL	0.207ns	0.103ns	-0.208ns	0.424**	0.119ns	0.679**	-0.010ns	0.360*	0.457**	0.521**			
SW	0.144ns	0.069ns	0.022ns	0.213ns	0.054ns	0.442**	0.095ns	0.178ns	0.160ns	0.244ns	0.705**		
SG	-0.029ns	-0.048ns	0.198ns	-0.233ns	-0.256ns	-0.179ns	0.367*	-0.042ns	-0.365*	-0.298ns	-0.350*	0.135ns	
NDVI	0.109ns	0.008ns	0.180ns	0.208ns	-0.231	0.577**	0.180ns	0.541**	0.216ns	0.345*	0.340*	0.326*	-0.07ns

Note: FPW: Fresh Pod Weight, DPW: Dry Pod Weight, 50DF: 50% days to flowering, HA: Harvest Age; PH: Plant Height; NTL: Number of Trifoliate Leaves; CD: Canopy Diameter; PL: Pod Length, PW: Pod Width; SL: Seed Length; SW: Seed Width; SG: Speed of Germination; NDVI: NDVI score at 9 WAP; *: Significant (p≤0.05); **: Highly significant (p≤0.01); ns: non-significant (p>0.05)

Discussion

The low similarity of 28% between genotypes from Indonesia and genotypes from Africa showed that there are many differences in morphological characteristics. According to De Kort et al. (2021), environmental and in particular climatic gradients are thought to affect population genetic diversity. This also causes differences in morphological characters in the 1st and 2nd clusters, as well as the 3rd and 4th clusters. High diversity can be utilized to create stable and superior cultivars.

Each cluster has different characteristics, some of which are negative, such as low yield in 2nd cluster. This could be due to infrequent rainfall during cultivation. For example, the Brown Gresik genotype in the previous study could produce 49.4 g dry pod weight per plant (Alhamdi et al. 2020). This indicates that 2nd cluster is a cluster that is sensitive to drought stress. The 3rd cluster (LunT and Tiga Nicuru) is unique because it has short plant characteristics, a small canopy, and fast maturity. The shortness of the plants in this cluster is not caused by drought stress but is due to their original character. This was proven by Khan et al. (2021) who stated that the average plant height of the bambara groundnut collection from Nigeria is 24.36 cm, almost the same as the lowest plant height in this study, namely Tiga Nicuru (24.33 cm). Tiga Nicuru genotype originated from Mali, West Africa, as did Nigeria. Mayes et al. (2019) stated that the Tiga Necuru genotype from Mali is described as a plant that is neutral to flowering time and sensitive to photoperiod for pod formation. This is a character that is suitable for development in dry areas.

Testa color is a qualitative characteristic that is quite noteworthy because it is a characteristic that differentiates one cultivar/genotype from another. Sari et al. (2021) revealed several bambara groundnut testa colors from Sukabumi (dark purple and deep purple) and Tasikmalaya (purplish brown, dark brown, deep purple, and blackish purple), but in this study, Sukabumi and Tasikmalaya were not classified at those colors. Agyeman et al. (2022) showed that some genotypes of bambara groundnut with a red seed coat color characterize a long planting period but have high productivity. On the contrary, a seed coat with a beige color has a short planting period but lower productivity. This statement is proven by DodR-R II, Uniswa R, and Uniswa R/G genotypes having high yields but long harvest ages, while LunT and Tiga Nicuru genotypes have short harvest ages with low yields.

Testa color can also affect germination. Fitriesa et al. (2016) reported that Sumedang's accession with purplish-black testa (seed coat) had low vigor (vigor index 1.8% and speed of germination 11.9%/day). This result is quite consistent with the results of our study that Black Sumedang genotype had a low germination percentage (12%) at 7 DAP (Table 6). Miya and Modi (2017) stated that the color pigments in some legume seeds can reduce water absorption, so seeds with dark testa germinate more slowly than those with light testa. This statement cannot be confirmed because the Tiga Nicuru and LunT genotypes have bright seed coats, but the LunT genotype was the slowest germination genotype, while the Tiga Nicuru genotype was the fastest (Table 6). Smykal et al. (2014) stated that some legume seeds' color pigments (dark color) contain tannin. The presence of tannin in the seed coat, especially near the hilum, has a role as an antifungal metabolite that needs further investigation.

The NDVI score measurement in this study did not affect the high yield produced. According to Hasan et al. (2018), plants with a deep green color indicated that the chlorophyll substance was high. In addition, Mikic et al. (2023) stated that NDVI is positively correlated with dry matter remobilization efficiency (DMRE) which is closely related to the drought-tolerant character. Logically, plants with higher chlorophyll content will produce higher nutrients for seeds. However, this did not happen to Black Madura genotype, which had a relatively low yield (22.87 g/plant of dry pod weight) but a high NDVI score (Figure 1). This is presumably due to genetic factors; not all genotypes can produce high pod weight, even though these genotypes are good at vegetative growth.

Flowering days were significantly positively correlated with plant height and number of leaves (Table 7). This indicated that the amount of vegetative growth will prolong the flowering time. The positive correlation between plant height and flowering time supports the results of Unigwe et al. (2016), which also showed a positive correlation, but different from the results of Khan et al. (2022), which showed a negative correlation between flowering time and vegetative growth variables (plant height and the number of branches); flowering days also had a very significant positive correlation with seed length (Table 7). There is an indication that the longer the bambara groundnut seed, the longer it takes to initiate flowering. The large seeds belong to the first cluster's genotypes, i.e., Sumedang and Sukabumi.

In this study, flowering time did not correlate with yield characteristics (fresh pod weight, dry pod weight, or number of pods per plant) (Table 7). However, the results of Khan et al. (2022) showed a negative correlation between flowering time with wet and dry pods and the number of pods per plant. This means that the faster the plants flower, the more pods they produce. However, it could be that the lack of pods in this study resulted from no watering, so the plants adapt by shortening their life span. This supports the statement of Feldman et al. (2019) that bambara groundnut is most impressive in terms of drought tolerance, encompassing all three composite traits of drought escape, avoidance, and tolerance.

The highest plant belongs to the genotypes in the first cluster, i.e., genotypes from Sumedang and Sukabumi, while the largest number of leaves belong to the genotypes in the second cluster, i.e., genotypes from Gresik, Madura, and Tasikmalaya (Figure 3). Plant height also had a very significant positive correlation with canopy diameter. This is natural because the taller the plant, the wider the canopy area. The diameter of the canopy also correlated with the number of leaves.

The NDVI variable had a very significant positive correlation with pod width, seed length, and seed width and was very significant with the plant height and canopy diameter (Table 7). A very significant correlation between plant height and canopy width is very reasonable because that was where the NDVI score was obtained. The length of the seed, the width of the seed, and the width of the pods were significantly positively correlated. The cause of this has not yet been found, but it could also be a feature that the greenness of the leaves is related to the size of the seeds (seed length and width). Gonne et al. (2013) stated that the earliness of flowering, number of pods per plant, and grain yield per plant were the most discriminant factors, suggesting their consideration when selecting agronomic superior traits. Seed length in this study correlated with many other morphological characters. This may need to be considered as a factor in breeding bambara groundnuts.

This study concludes that the 16 bambara groundnut genotypes can be classified into four clusters. The first cluster belongs to Sumedang and Sukabumi, the second belongs to Gresik, Madura, and Tasikmalaya, the third comes from West Africa, and the fourth comes from East Africa and Southern Africa. The NDVI score measurement in this study did not affect the yield produced. Tiga Nicuru, DodR-R II, M-14 Gresik, Black Sukabumi, and Black Madura were the genotypes with the highest germination speed, while the LunT genotype was the lowest.

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