

Research Article

Genetic Diversity Estimated By Morphological Descriptors of Yam Accessions

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Abstract: The collection of yam germplasm (*Dioscorea* spp.), maintained by the Universidade Federal do Recôncavo Baiano was gathered through collection expeditions in different areas of the Brazilian northeast. Research on plant genetic resources is essential for the conservation of genetic diversity and to study the divergence between accessions, which is the basis for breeding programs. To understand the morphological variability maintained in this germplasm, we evaluated 28 morphological characteristics of 38 accessions of yam. The data allowed us to estimate the entropy level of the characters evaluated and the genetic dissimilarity of the yam accessions. The individuals were then grouped by the Unneeded Pair Group Method with Arithmetic Mean (UPGMA) grouping method. The yam accessions studied were divided into four distinct groups. The hierarchical clusters, which are accessions grouped according to the botanical species (*D. trifida*, *D. rotundata*, *D. cayenensis*, *D. alata* and *D. bulbifera*), showed a high genetic variability between the groups and a reduced variability within each group. The value of the cophenetic correlation coefficient between the grouping matrix and the distance matrix was highly significant (0.92). Some characters presented with intermediary entropies while most had low values, suggesting reduced variability. The data calls for greater efforts in yam prospection involving more individuals and locations. Further, this study revealed the greatest entropies for the traits stem diameter and number and shape of the tubers, which showed high variability.

Keywords: *Dioscorea*, Dissimilarity, Entropy, Variability, Germoplasm.

INTRODUCTION

Yam (*Dioscorea* spp.) is one of the most important root and tuber crop in the world (SILVA *et al.*, 2017). The Dioscoreaceae are distributed throughout the tropical, subtropical, and temperate regions of the world (ANDRES *et al.*, 2017). The global yam cultivation area in recent year's amounts 7.5 million hectares, generating 65.9 million tons of tubers with an average crop yield of 8.85 t ha⁻¹. In Brazil, yam crops occupy 25.889 ha generating 250.340 ton of tubers with an average yield of 9.67 t ha⁻¹ (FAOSTAT, 2016). However, it is estimated that the crop has the potential to produce 30 t ha⁻¹ under suitable growing conditions.

In Brazil, yam is one of the most cultivated crops by small family farmers playing an important socioeconomic role especially in northeastern part of

the country, where more than 90% of the Brazilian production of edible species of yam takes place (MENDES *et al.*, 2013). The crop is ideally positioned to be cultivated in the region due to its favorable edaphoclimatic conditions and the potential expansion of the cultivation area. Therefore, yam has a great potential to foster economic development of the Brazilian northeast driven by the large consumer centers of the Brazil and abroad.

In spite of the potential that the yam culture could represent for the Brazilian northeast agribusiness, its productivity remains low in comparison to the production performance of African countries such as Nigeria, which leads the global production of the crop with 44 million tons in 2016 (ANDRES *et al.*, 2017; FAOSTAT, 2016). Among the factors underlying this low productivity are the use of seed pods of lower

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agronomic quality (non-uniformity) in size and maturation, contamination by nematodes, fungi, and viruses as well as low soil fertility, climatic irregularities, and inadequate crop, soil, and water managements (BRITO *et al.*, 2011; NASCIMENTO *et al.*, 2015).

Therefore, the development of new disease-resistant and higher-quality yam cultivars is warranted. However, breeding is only possible if genetic variability is available. To meet this condition, it is important to preserve yam's genetic resources and to this end, one of the most important steps is the establishment of a collection of accessions through the implementation of a germplasm bank. However, the proper use of a species' germplasm is only possible if a deeper knowledge about the accessions is achieved by means of morphological and molecular characterization.

Thus, it is justified the implantation, characterization, and conservation of a collection of accessions of yam to gather and maintain genetic variability and to search for genotypes more adapted to the environment that could meet the needs of farmers and the current market (LOPLOPEZ-MONTES, 2012).

Studies involving plant divergence have been carried out involving botanical, morphological, agronomic, and molecular descriptors (SHEELA *et al.*, 2016) However, interpretations of these data have usually been made by univariate analyzes, which creates difficulties to obtain estimates of divergence and, consequently, to select individuals that are desirable for cross-linking since differences between groups or populations may not depend on a single variable but rather a set of them (CRUZ *et al.*, 2012).

It was aimed to characterize *Dioscorea* spp. accessions of the Yam Germplasm Bank maintained by the Universidade Federal do Recôncavo Baiano (UFRB) by means of morphological descriptors to detect the genetic diversity needed to assist breeding programs for this crop.

MATERIAL AND METHODS

The Yam Germplasm Bank of UFRB was implanted in the Experimental Campus of Cruz das Almas, in the state of Bahia, northeast of Brazil. Thirty-eight accessions of yams (Table 1) were evaluated using 28 descriptors: 10 related to leaves, seven to stem, and 11 to the tubers (Table 2).

Table 1 – List of accessions of *Dioscorea* spp. maintained at the Yam Germplasm Bank of the Universidade Federal of Recôncavo Baiano, Brazil.

Nº	Accessions	Species	Place of seed purchase	Local of origin
1	BGIN-85	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Toá Santo Antônio da Jaqueira – BA
2	BGIN-86	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Sítio São Roque – São Felipe – BA
3	BGIN-87	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Faz. Batatan Maragogipe – BA
4	BGIN-88	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	St. Antônio da Jaqueira São Félix–BA
5	BGIN-89	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Boa Vista São Félix – BA
6	BGIN-90	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Engenho de São João – BA
7	BGIN-91	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Guaripa Maragogipe – BA
8	BGIN-92	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Faz. Vendinha São Felipe – BA
9	BGIN-93	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Faz. Dois Irmãos Cruz das Almas–BA
10	BGIN-94	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Cruz das Almas – BA
11	BGIN-95	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	São Felipe – BA
12	BGIN-96	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Maragogipe – BA
13	BGIN-97	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Cruz das Almas – BA
14	BGIN-98	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Cruz das Almas – BA
15	BGIN-100	<i>D. rotundata</i>	Feira de Cruz das Almas-- BA	Cruz das Almas – BA
16	BGIN-101	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Cruz das Almas – BA
17	BGIN-102	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Faz. Copioba São Felipe – BA
18	BGIN-104	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Faz. Sapezinho São Felipe – BA
19	BGIN-105	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Piedade – Maragogipe – BA
20	BGIN-107	<i>D. cayenensis</i>	Feira de Cruz das Almas - BA	Combê – Cruz das Almas – BA
21	BGIN-108	<i>D. alata</i>	Feira de Cruz das Almas - BA	Cruz das Almas – BA
22	BGIN-110	<i>D. rotundata</i>	Feira de Cruz das Almas - BA	Maragogipe – BA
23	BGIN-111	<i>D. alata</i>	Feira de Cruz das Almas - BA	Serraria – Maragogipe – BA
24	BGIN-112	<i>D. rotundata</i>	Feira de Juazeiro - BA	Caruaru – PE
25	BGIN-113	<i>D. alata</i>	Feira de Juazeiro - BA	Caruaru – PE
26	BGIN-115	<i>D. alata</i>	Feira de Juazeiro - BA	Caruaru – PE
27	BGIN-116	<i>D. rotundata</i>	Feira de Juazeiro- BA	João Pessoa - PB
28	BGIN-117	<i>D. alata</i>	Feira de Juazeiro - BA	João Pessoa - PB
29	BGIN-118	<i>D. bulbifera</i>	Feira de Cruz das Almas - BA	Maragogipe – BA
30	BGIN-119	<i>D. rotundata</i>	Feira de Santana - BA	Maragogipe - BA
31	BGIN-120	<i>D. bulbifera</i>	Cruz das Almas - BA	Maragogipe – BA
32	BGIN-121	<i>D. rotundata</i>	Cruz das Almas - BA	Batatan – Poerinha – BA

33	BGIN-125	<i>D. trifida</i>	Feira de Valença - BA	Orobó Valença – BA
34	BGIN-131	<i>D. rotundata</i>	Feira de Nazaré - BA	Maragogipe – São Felipe – BA
35	BGIN-133	<i>D. rotundata</i>	Supermercado de Campina Grande – PB	Região de Remijo – PB
36	BGIN-135	<i>D. rotundata</i>	Supermercado de Campina Grande – PB	Região de Remijo – PB
37	BGIN-136	<i>D. rotundata</i>	Supermercado de Campina Grande – PB	Região de Remijo – PB
38	BGIN-137	<i>D. rotundata</i>	Supermercado de Campina Grande – PB	Região de Remijo – PB

BA: State of Bahia; PE: State of Pernambuco; PB: State of Paraíba. All these states are located in the northeast region of Brazil.

Samples used to evaluate the morphological descriptors followed the information listed in the catalog of descriptors of the International Plant Genetic Resources Institute (IPGRI) and the International Institute of Tropical Agriculture (IITA) (IPGRI / IITA, 1997) (Table 2).

Table 2 - Descriptors used for the morphological characterization of *Dioscorea* spp.

Descriptors	Phenotypical classes
Stem color	1- Green; 2- Green with purple bands ; 3- Green with brown bands, and 4- Purple
Presence of wings	1-Present; 2-Absent
Color of wings	1-Green; 2-Purple
Presence of aculeus	1-Present; 2-Absent
Direction of growth	1-Clockwise; 2-Counter-clockwise
Stem diameter (15 cm from the base)	1- < 0.4 cm; 2- 0.4 – 0.6 cm; 3- >0.6 cm
Stem shape (cross section)	1-Poliyonal; 2-Round
Position of the leaves	1-Alternate; 2-Opposite
Shape of the leaves	1-Cordate; 2-Sargitate
Number of leaf lobes	1-One; 2-Three
Color of the leaves	1-Green; 2-Dark green; 3-Purple
Length of the petiole	1- < 5 cm; 2- 5- 10 cm; 3- > 10 cm
Color of the Petiole	1- Green; 2- Green and brown; 3- Purple
Width of the leaf at the largest section	1- < 10 cm; 2- 10 – 15 cm; 3- >15 cm
Flowering	1-Present; 2-Absent
Underground tubers	1-Present; 2-Absent
Presence of aerial rhizophores	1-Present; 2- Absent
Distance between the insertion of the petiole on the upper end of leaf (adult leaves)	1- < 2 cm; 2- 2 – 4 cm; 3- > 2 cm
Distance between the insertion of the petiole in the leaf to the lower end of the leaf (adult leaves)	1- < 10 cm; 2- 10 – 15 cm; 3- >15 cm
Presence of roots in rhizophores	1-Present; 2-Absent
Number of tubers	1- One; 2-Some; 3-Many
Shape of the tubers	1- Elongated; 2-Irregular; 3-Oval
Position of the junction between the tubers	1- All; 2-Superior
Length of the tubers	1- <20 cm; 2- 20-40 cm; 3- >40 cm
Width of the tubers (major axis)	1- <7 cm; 2- 7-12 cm; 3- >12 cm
Mass of the tubers	1- <0.70 kg; 2- 0.70-1.50 kg; 3- 1.60-2.0 kg; 4- 2.10-3.0 kg; 5- >3.0 kg
Peel color	1- Brown; 2- Yellow
Flesh color	1-White; 2-Yellow; 3-Purple; 4-Purple and white; 5-White and Purple

After 4-5 months of establishing the germplasm, the aerial part (leaves and stems) was characterized with the aid of a measuring tape and pachymeter. Nine months later we evaluated the tubers using a tape measure and a scale.

Based on the field data, the entropy level of the characters was estimated using the Shannon-Weaver (H') diversity coefficient (SHANNON; WEAVER, 1949) by the SAS program (SAS / STAT SOFTWARE, 2006).

The greater the number of phenotypic classes and the greater the entropy of a given descriptor, the more homogeneous is the balance between the

frequency of accessions in the different phenotypical classes. In other words, for a morphological descriptor with two phenotypic classes, the greatest entropy will occur when both classes present 50% of the accessions evaluated (VIEIRA *et al.*, 2008).

The distance matrix was generated using the R software (R DEVELOPMENT TEAM, 2016). The hierarchical groupings generated from the genetic distance matrix were obtained by the UPGMA method (Unneeded Pair Group Method with Arithmetic Mean) (SNEATH; SOKAL, 1973) using the GENES program (CRUZ, 2013). Next, based on the genetic dissimilarity matrix, the clusters were validated by means of the

cophenetic correlation coefficient (r) (SOKAL; ROHLF, 1962). The significance of the cophenetic correlation coefficient was calculated by the Mantel test (1967) with 1,000 permutations. The dendrogram was generated using the STATISTICA 7.0 program (STATISTICA, 2005).

RESULTS AND DISCUSSION

The yam accessions were implanted in the experimental field of UFRB and characterized by morphological descriptors. Based on Jamago (2003), the Shannon-Weaver (H') coefficient values are

classified as low (H' <0.50), medium or intermediate (H' = 0.50-0.75), and high (H' ≥ 0.75). Thus, the variables that presented low entropy were: presence of wings, color of wings, stem shape, leaf position, leaf shape, number of leaf lobes, color of petiole, distance between the petiole insertion in the leaf at the upper end (adult leaves), distance between the insertion of the petiole in the leaf to the lower end of the leaf (adult leaves), presence of aerial rhizophores, presence of roots in the rhizophores, position of the junction between the tubers, length of the tubers, width of the tubers, and pulp color (Table 3).

Table 3 - Descriptive statistics for the quantitative descriptors used in the characterization of the Yam Germplasm Bank of the Universidade Federal do Reconavo Baiano, Brazil.

Descriptor	Classes	Frequency %	Entropy level
Stem color	Green	84.21	0.51
	Green with purple stripes	2.63	
	Green with Brown stripes	13.16	
Presence of wings	Presence	15.79	0.44
	Absence	84.21	
Color of wings	Green	84.21	0.44
	Purple	15.79	
Presence of aculeus	Presence	76.32	0.55
	Absence	23.68	
Stem diameter	< 0.4 cm	50	0.8
	0.4 – 0.6 cm	47.37	
	> 0.6 cm	2.63	
Stem form	Polygonal	15.79	0.44
	Round	84.21	
Position of the leaves	Alternate	18.42	0.48
	Opposite	81.58	
Leaf shape	Cordate	5.26	0.33
	Sagitate	92.11	
	Lobulate	2.63	
Number of leaf lobes	One	97.37	0.12
	Three	2.63	
	Green	34.21	
Dark green	65.79		
Petiole length	< 5 cm	13.16	0.59
	5 – 10 cm	81.58	
	> 10 cm	5.26	
Petiole color	Green	86.84	0.46
	Green with brown	10.53	
	Purple	2.63	
Distance between the insertion of the petiole in the leaf to the superior end of the leaf (adult leaves)	2 – 4 cm	13.16	0.39
	> 2 cm	86.84	
Distance between the insertion of the petiole in the leaf to the end of the leaf (adult leaves)	< 10 cm	2.63	0.24
	10 – 15 cm	94.74	
	> 15 cm	2.63	
Width of the leaves (major axis)	< 10 cm	44.74	0.69
Continued...	10 – 15 cm	55.26	
Presence of aerial rhizophores	Presence	5.41	0.21
	Absence	94.59	
Presence of roots in rhizophores	Presence	5.41	0.21
	Absence	94.59	
Number of tubers	One	8.11	0.86
	Some	62.16	

	Many	29.73	
Tuber shape	Alongated	54.05	0.94
	Irregular	10.81	
	Oval	35.14	
Position of the junction between the tubers	All	5.41	0.21
	Superior	94.59	
Tuber length	< 20 cm	91.89	0.28
	20 - 40 cm	8.11	
Tuber width (major axis)	7 -12 cm	5.41	0.21
	> 12 cm	94.59	
Tuber weight	< 0.70 Kg	91.89	0.28
	0.70 – 1.50 Kg	8.11	
Peel color	Brown	29.73	0.61
	Yellow	70.27	
Pulp color	White	89.19	0.34

The entropy level can be used to quantify the variability present in the qualitative descriptors by observing the relative frequencies of the classes for each descriptor evaluated. According to Ledo *et al.*, (2011), low values for entropy are associated to a lower number of phenotype classes for the descriptor and a greater imbalance in the proportion between the frequency of accessions in the different phenotypic classes.

The variables that presented intermediate values of entropy were: stem color, presence of aculeus, leaf color, petiole length, leaf width (major axis), and peel color. The highest values of entropy were observed for: stem diameter and the number and shape of tubers. These high entropy values were observed in a great number of classes. They also presented a more balanced frequency of accessions in the different phenotypic classes, at least in more than 50% of them, suggesting a genetic variability among the accessions (Table 3). The other 16 variables presented low entropy classes with $H' < 0.50$. Thus, the present study revealed genetic variability among the studied accessions, mainly for medium and high entropy variables, albeit for a small number of phenotypic classes.

Menisa (2008) verified high genetic diversity among the accessions for *D. alata* and *D. esculenta* using the Shannon-Weaver index. Studying a yam collection in Bangladesh, Islam *et al.*, (2011) found that the following descriptors presented the largest entropies: petiole color (0.94), presence of aerial tubers (0.79), tuber shape (0.91), and pulp color (0.85). The analyses of morphological variability of 91 accessions of the *D. cayenensis-rotundata* complex in Ghana showed high entropy values for the number of shoots, stem diameter, stem length and width, number of tubers per plant, tuber width, number of inflorescences per plant (OTOO *et al.*, 2009). Researching *Manihot esculenta*, Vieira *et al.*, (2008) found the greatest entropies for external stem color, petiole color, central lobe shape, and color of the apical leaf. The

morphological characterization of the *Manihot* spp of Embrapa Mandioca and Fruticultura revealed high levels of entropy for petiole color, lobe shape, external stem color and number of lobes (LEDO *et al.*, 2011).

The dendrogram of dissimilarity for this study was constructed with 25 descriptors in 38 accessions of yam (Figure 1).

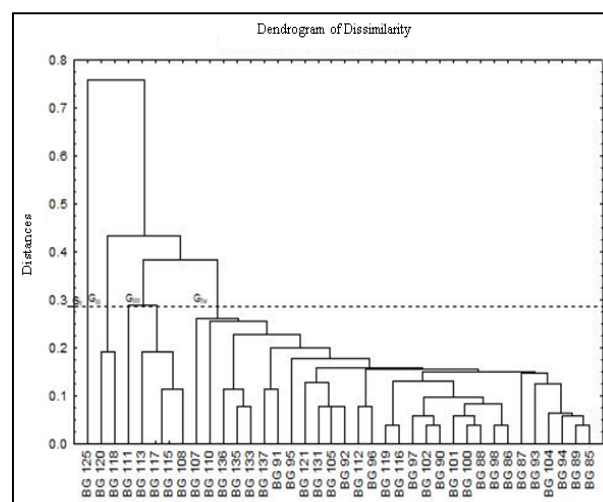


Figure 1 - Genetic dissimilarity among 38 yam genotypes based on 26 morphological descriptors using the Cole-Rodgers Similarity Coefficient and the UPGMA clustering method.

Because the variables direction of growth, flowering, and the presence of subterranean tubers did not discriminate variations among the evaluated accessions, we excluded them from the analyses. The grouping based on the UPGMA method identified four groups and revealed the mean value of the grouping matrix (0.26), which was the method that best grouped the accessions (Table 4). The largest group was the fourth, which assembled 79.8% of the accessions, followed by group 3 (13.1%), group 2, (5.25%), and group 1 with only one representative (2.63%).

Table 4 - Grouping of yam accessions using the UPGMA method.

Groups	Accessions
1	BG125
2	BG120, BG118
3	BG111, BG113, BG117, BG115, BG108
4	BG107, BG110, BG136, BG135, BG133, BG121, BG137, BG91, BG97, BG102, BG90, BG119, BG116, BG95, BG93, BG87, BG101, BG100, BG88, BG98, BG86, BG112, BG96, BG131, BG105, BG92, BG104, BG94, BG89, BG85

The hierarchical grouping combined the accessions according to the botanical species (*D. trifida*, *D. rotundata*, *D. cayenensis*, *D. alata* and *D. bulbifera*). This was probably because these characteristics are mostly qualitative and, therefore, controlled by few genes and slightly affected by the environment (FALCONER, 1981) that can discriminate yam accessions according to the species (MARTINELLO *et al.*, 2001).

The dendrogram satisfactorily distinguished the groups, albeit with a lower ability to differentiate the accessions within the formed groups, suggesting a reduced genetic variability within each species. The results also revealed no association between genetic diversity and eco-geography of the accessions. Similar results were observed by Sartie *et al.*, (2012) while studying 60 accessions of *Dioscorea* spp. (*D. alata* L., *D. cayenensis* Lam., *D. dumetorum* (Kunth) and *D. rotundata* Poir). Regarding the phenotypic diversity of 23 morphological characteristics, the authors verified that there is a greater variability among the species than within the species. Norman *et al.*, (2011) characterized the morphological variability of 52 accessions of *Dioscorea* sp. analyzing 28 characteristics, by means of Principal Component Analysis and cluster analysis. They assembled six groups with genetic divergence of yam cultivars that could be used for the development of high yield genotypes and other desired characteristics such as resistance to pests and local diseases.

The value of the cophenetic correlation coefficient, which measures the degree of fitness between the dissimilarity matrix and the resulting matrix of simplification provided by the cluster, was $r = 0.92^{**}$, a highly significant value. A previous study by Sartie *et al.*, (2012), characterizing the genetic diversity of *Dioscorea* spp. by morphological descriptors, obtained a cophenetic correlation coefficient of $r = 0.67$, which is considered both high and adequate. Indeed, Vaz Patto *et al.*, (2004) suggested that $r > 0.56$ is considered ideal and reflects a good agreement between the dissimilarity matrix and the clustering matrix.

CONCLUSION

Character analysis using the Shannon-Weaver diversity index is an efficient strategy to evaluate the dissimilarity between *Dioscorea* spp. We found intraspecific genetic divergence between *Dioscorea* spp. in the Yield Germplasm Bank of the UFRB but reduced variability within each species. Therefore, it further yam prospecting efforts are necessary in order to

collect more individuals in different locations to increase variability and expand the possibilities for breeding programs of the yam species.

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