

Full Length Research Paper

Genetic variability of yam based on quantitative descriptors

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Received 16 February, 2018; Accepted 13 March, 2018

Yam is a promising income source for small producers in the Recôncavo Baiano region. However, few genetic studies have been conducted with this culture. In this sense, the aim of this study was to assess genetic variability among 89 genotypes of yam (*Dioscorea rotundata* Poir.) collected in four municipalities in Bahia State, Brazil. These were assessed based on eight quantitative descriptors. Data were subjected to descriptive statistics, Spearman's rank correlation, Singh criterion, cluster analysis, principal component analysis and dispersion graph. The tuber weight showed the highest coefficient of variation and correlated positively and significantly with width and length of tuber. The tuber length characteristics contributed most to the genetic divergence. The criterion pseudo- t^2 divided 89 genotypes into seven groups, being that groups 4, 5 and 7 showed the highest averages for the production characteristics. The first three principal components explained 69.49% variation and dispersion graph showed partly concordant with UPGMA method. This study revealed the existence of genetic variability of yam, which may be explored in future genetic improvement programs.

Key words: *Dioscorea rotundata* Poir., multivariate methods, plant genetic resources, plant breeding.

INTRODUCTION

Yam (*Dioscorea rotundata* Poir.) is one of 600 species of Dioscoreaceae family, native from Africa and distributed through tropical and subtropical world regions (Lebot, 2009). It is a diploid plant ($2n=2x=40$) (Scarcelli et al., 2005), monocotyledon, dioecious and propagated vegetative, which produces tubers rich in carbohydrates (Lebot, 2009), which confer great economic importance.

The yam production in Brazil in 2014 was about 247,000 tons cultivated on 25,000 hectares (FAO, 2016). The largest production is concentrated in Northeast, where *D. rotundata* and *Dioscorea cayenensis* occupy 90% of production (Silva et al., 2016). Bahia State is the third-largest producer and Recôncavo region represents the largest cultivated area, including Maragogipe, São

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Table 1. Code, village, municipalities, geographic coordinates and number of 89 yam (*Dioscorea rotundata*) genotypes, collected in the Recôncavo da Bahia region (Cruz das Almas, BA, Brazil, 2016).

| Code | Origin | | | Number | |
|---------|------------------|----------------|----------|----------|-----------|
| | Village | Municipality | Latitude | | Longitude |
| 1 | Tua | Cruz das Almas | 12°43' S | 39°03' W | 1 |
| 2 | Cadete | Cruz das Almas | 12°42' S | 39°04' W | 1 |
| 3 | Três Boca | Cruz das Almas | 12°43' S | 39°04' W | 1 |
| 4 - 9 | Camargo | São Felipe | 12°44' S | 39°03' W | 6 |
| 10 - 15 | Jaracandá | São Felipe | 12°44' S | 39°03' W | 6 |
| 16 - 21 | Bom Gosto | São Felipe | 12°45' S | 39°03' W | 6 |
| 22 - 27 | Boa Esperança | São Felipe | 12°45' S | 39°03' W | 6 |
| 28 - 33 | Campo das Flores | São Felipe | 12°46' S | 39°02' W | 6 |
| 34 - 39 | São Bento | São Félix | 12°41' S | 39°02' W | 6 |
| 40 - 45 | Engenho São João | São Félix | 12°42' S | 39°02' W | 6 |
| 46 - 51 | Monte Alegre | São Félix | 12°40' S | 39°02' W | 6 |
| 52 - 57 | Boa Vista | São Félix | 12°42' S | 39°02' W | 6 |
| 58 - 63 | Matatauba | São Félix | 12°41' S | 39°02' W | 6 |
| 64 - 65 | Serraria | Maragogipe | 12°44' S | 39°00' W | 2 |
| 66 - 71 | Campinas | Maragogipe | 12°45' S | 39°02' W | 6 |
| 72 - 77 | Encruzilhada | Maragogipe | 12°44' S | 38°54' W | 6 |
| 78 - 89 | Batatans | Maragogipe | 12°44' S | 39°02' W | 12 |
| Total | - | - | - | - | 89 |

Felipe, São Felix and Cruz das Almas municipalities.

Despite its importance to family farming, yam has suffered biotic and abiotic stresses caused by fungal diseases, lack of propagative material of excellent quality, that threaten the genetic variability of these genetic resources and consequent culture abandonment. In this sense, it is necessary to conduct studies aiming at characterizing genetic variability existing in species of *Dioscorea* species genus, to identify superior genotypes to develop improved varieties that combine resistance to diseases and pests, and high productivity with attributes appreciated by consumers (Obidiegwu et al., 2009), such that contribute in maintaining sustainability of this culture agribusiness.

The characterization and assessment of genetic variability are fundamental activities in genetic improvement programs for several species of agronomic interest. However, these depend on the use of multivariate statistical methods. The principal component analysis, canonical variable analysis and cluster analysis are the most publicized techniques among breeders (Mohammadi and Prasanna, 2003; Sudré et al., 2007). The choice of method depends on research objectives and hypothesis to be tested (Mingoti, 2007), as well as the desired accuracy by researcher, ease of analysis and the way in which data were collected (Cruz et al., 2012). Among the many available clustering methods, unweighted pair group method with arithmetic mean (UPGMA) has been the most used, due to the present higher values of cophenetic correlation coefficients, which

measure the association between dissimilarity matrix and the cluster matrix (Mohammadi and Prasanna, 2003). For this reason, this method has been used in several studies that aim to characterize and assess the genetic variability of yams, such as those by Bressan et al. (2014) and Efiue (2015).

However, in other studies of genetic variability characterization of yams, UPGMA method has been used simultaneously with principal components and both are complemented by graphic dispersion analysis (Mwirigi et al., 2009; Norman et al., 2011; Michael, 2013; Mulualem and Michael, 2013; Siadjeu et al., 2015). These techniques are used in many studies of genetic variability characterization of yams (Mwirigi et al., 2009; Norman et al., 2011; Mulualem and Michael, 2013; Bressan et al., 2014; Efiue, 2015; Siadjeu et al., 2015). Thus, this study aimed to assess the genetic variability among genotypes of yam (*D. rotundata*), collected in four municipalities from the Recôncavo Baiano region, based on quantitative descriptors through multivariate methods.

MATERIALS AND METHODS

A total of 89 genotypes of yam were collected in 18 rural properties of villages belonging to the municipalities of Cruz das Almas, São Félix, São Felipe and Maragogipe, which are located in the region of Recôncavo Baiano. Collection points were georeferenced through global positioning system (GPS) (Table 1).

Genotype characterization was divided in two stages. First step took place in May and June 2015, in which four expeditions were conducted to villages in municipalities mentioned earlier (Table 1).

Table 2. Descriptive statistics and normality test of variables analyzed in 89 yam genotypes, Cruz das Almas, BA, Brazil, 2016.

| Variable | Minimum | Maximum | Average | Standard deviation | CV (%) | Normality test |
|----------|---------|---------|---------|--------------------|--------|--------------------|
| PL (cm) | 3.00 | 7.60 | 4.92 | 1.03 | 20.92 | 0.97 ^{ns} |
| TL (cm) | 14.10 | 39.30 | 25.19 | 5.74 | 22.80 | 0.97* |
| SD (mm) | 0.20 | 0.60 | 0.43 | 0.10 | 23.62 | 0.91** |
| F1 (cm) | 4.00 | 11.30 | 6.41 | 1.60 | 24.98 | 0.95** |
| TW (cm) | 4.40 | 12.00 | 7.06 | 1.54 | 21.84 | 0.97* |
| P2 (cm) | 9.30 | 19.80 | 14.34 | 1.93 | 13.46 | 0.99 ^{ns} |
| P3 (cm) | 1.90 | 8.00 | 3.93 | 1.24 | 31.56 | 0.96** |
| WT (kg) | 0.32 | 3.16 | 0.81 | 0.42 | 51.56 | 0.77** |

**Significant 1% probability. *Significant 5% probability. ^{ns}Not significant at 5% probability by Shapiro-Wilk test.

In each rural property, six genotypes were selected at random, except in properties from Tua, Cadete and Três Boca, where only one genotype was selected and Serraria village, with two genotypes (Table 2). The genotypes were marked with a tape, which represented their identification (1 to 6), shoot (leaves and stems) were characterized using measuring tape and caliper. The second stage was made in December 2015 (two expeditions), where tubers were collected from genotypes, previously marked, bagged and transported to Food Technology Laboratory of Federal University of Recôncavo da Bahia (UFRB), and characterized with measuring tape and a scale.

The genotypes were characterized based on eight quantitative descriptors established by International Plant Genetic Resources Institute and International Institute of Tropical Agriculture (IPGRI/IITA, 1997): stem diameter (SD) in mm measured at 15 cm from ground, petiole length (PL) in cm, leaf width at the widest portion in cm (F1), distance among the petiole insertion in the leaf, the upper end (P3) and lower end of the leaf (P2) in cm, tuber length in cm (TL), tuber width in cm (TW) and tuber weight in kg (WT). Data of descriptors SD, PL, F1, P3 and P2 were collected at 90 days after planting (DAP) during vegetative growth of the plants, whereas TL, TW and WT were collected at 270 DAP when tubers were harvested.

Data values were introduced in a database and descriptive statistics: minimum and maximum values, average, standard deviation and coefficient of variation were calculated. Shapiro-Wilk test for normality was used at 5% of probability.

Genetic variability was estimated by means of cluster analysis and principal component analysis. For cluster analysis, the average Euclidean distance was used as a measure of dissimilarity from standardized data. The relative contribution of characters was determined by the method proposed by Singh (1981). The hierarchical clusters from the distance matrix were obtained by UPGMA method (unweighted pair-group method using arithmetic averages) (Sneath and Sokal, 1973). Validation of clusters was determined through the cophenetic correlation coefficient (r) (Sokal and Rohlf, 1962). The significance of the cophenetic correlation coefficient was calculated by the Mantel (1967) test with 1,000 permutations. The criterion for defining the cluster number was based on pseudo-t² index from package "NbClust" of R software (Charrad et al., 2014). Statistical analyses were made using GENES version 5.1 (Cruz, 2013) and R program version 2.2-6 software (R Core Team, 2014).

RESULTS AND DISCUSSION

Descriptive statistics for eight descriptors of 89 yam genotypes under study are shown in Table 2.

F1, Leaf width at widest portion; P2 and P3, distance among petiole insertion in leaf, the lower and upper leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter; WT, tuber weight.

The variation coefficient (CV) varied between 13.46 and 51.56%. The variables with the highest variation coefficients were WT and P3 with 51.56 and 31.56%, respectively. On the other hand, P2 presented the lowest variation coefficients with 13.46%. TL ranged from 14.1 to 39.3 cm and TW ranged 4.4 to 12.0 cm with an average of 7.06 cm. These results agree with those obtained by Anokye et al. (2014), in *Dioscorea alata* for TW, F1 and WT. In general, it was observed that production variables (weight, width and length of tuber) showed variability, expressed by their variation coefficient (CV). This variability can be exploited in improvement programs, since these features constitute the main objective in commercial exploitation of yams (Domingos et al., 2014).

The normality test revealed that SD, F1, P3 and WT were ($p < 0.01$) by Shapiro-Wilk test. On the other hand, TL and TW were ($p < 0.05$) by the same test, indicating that these variables do not follow normal distribution; therefore, Spearman's rank correlation was determined among variables.

Association among variables gives an indication to carry out an effective breeding program (Tena et al., 2016) being a useful tool for indirect selection. Table 3 shows Spearman's rank correlation coefficients among variables. The PL showed positive correlation ($p < 0.01$) with the F1 ($r_s = 0.64$), with P2 ($r_s = 0.51$) and P3 ($r_s = 0.30$). It was also observed that WT was positively correlated ($p < 0.01$) with the TW ($r_s = 0.70$) and TL ($r_s = 0.45$), indicating that increase of WT leads to increase of TW and TL consequently. Highly significant correlation among characters indicates the possibility of simultaneous genetic improvement among them (Osuagwu et al., 2014).

F1, leaf width at the widest portion; P2 and P3, distance among petiole insertion in leaf, the lower and upper leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter, WT, tuber weight.

According to Singh (1981) method, TL with 74.24%

Table 3. Spearman's rank correlation coefficient, with the respective significance of the analyzed variables (Cruz das Almas, BA, Brazil, 2016).

| Variable | TL | SD | F1 | TW | P2 | P3 | WT |
|----------|--------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| PL | -0.24* | 0.14 ^{ns} | 0.64** | 0.02 ^{ns} | 0.51** | 0.30** | -0.06 ^{ns} |
| TL | | 0.02 ^{ns} | -0.20 ^{ns} | -0.02 ^{ns} | -0.21 ^{ns} | -0.03 ^{ns} | 0.45** |
| SD | | | 0.05 ^{ns} | 0.17 ^{ns} | 0.10 ^{ns} | -0.20 ^{ns} | 0.06 ^{ns} |
| F1 | | | | -0.16 ^{ns} | 0.48** | 0.37** | -0.20 ^{ns} |
| TW | | | | | -0.09 ^{ns} | -0.19 ^{ns} | 0.70** |
| P2 | | | | | | 0.21* | -0.25* |
| P3 | | | | | | | -0.18 ^{ns} |

**Significant 1% probability. *Significant 5% probability. ^{ns}Not significant at 5% probability by t test.

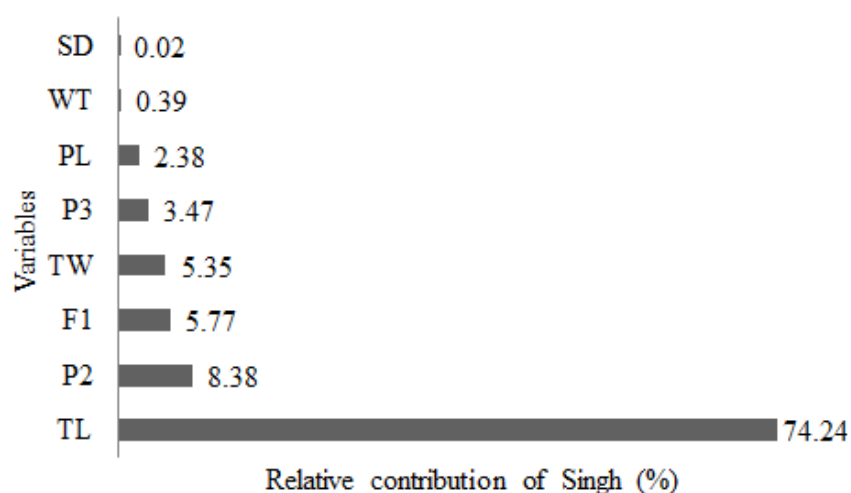


Figure 1. Relative contribution of the characters to diversity according to Singh (1981). F1, Leaf width at widest portion; P2 and P3, distance among petiole insertion in leaf, the lower and upper of the leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter; WT, tuber weight.

was the variable that showed the highest contribution to genetic dissimilarity among analyzed genotypes (Figure 1). In this sense, this variable must be prioritized to efficient choice of promising materials. However, Afonso et al. (2014) evaluating 209 yam genotypes, found that tuber length had less contribution, diverging this study. This difference is probably due to conditions management practiced by the producers. On the other hand, variables with little contribution were SD and WT, with 0.02 and 0.39%, respectively.

Cophenetic correlation coefficient (CCC) was 0.72, appropriate value according to Vaz Patto et al. (2004), considered ideal ($r \geq 0.56$), reflecting a good agreement between the distance of the dissimilarity matrix and cluster matrix.

Based on pseudo- t^2 index criterion, 89 yam genotypes were divided into seven clusters, by UPGMA method (Table 4). The number of clusters obtained in this study

was superior to those found by Beyene (2013), Bressan et al. (2014) and Siadjeu et al. (2015), thus revealing genetic variability existence of *D. rotundata* in Recôncavo Baiano region.

Group 3 combined the largest number of genotypes (39), being 16 from São Félix, 14 from São Felipe and 9 from Maragogipe. Group 2 were allocated 26 genotypes, 14 originating from Maragogipe, 7 from São Félix, 3 from São Felipe and two from Cruz das Almas. In turn, the group 1 consisted of 12 genotypes, 8 from São Felipe, 3 from São Félix and 1 from Cruz das Almas. Group 6 encompassed 4 genotypes, 3 collected in Maragogipe and the other in São Felipe. The groups 4 and 5 were composed of 3 genotypes, respectively, being that the group 4 included genotypes from São Felipe, while the group 5 was formed by two, which were obtained from São Félix and the other from São Felipe. The group 7 consisted of only two genotypes from São Félix.

Table 4. Cluster of 89 genotypes of yam (*Dioscorea rotundata* Poir.) collected in four municipalities of Recôncavo Baiano, in accordance with UPGMA method, based on eight quantitative descriptors, Cruz das Almas, BA, Brazil, 2016.

| Cluster | Number of genotypes | Genotypes |
|---------|---------------------|---|
| 1 | 12 | 1, 4, 5, 9, 10, 12, 15, 16, 17, 37, 40, 47 |
| 2 | 26 | 2, 3, 25, 27, 33, 35, 42, 44, 45, 51, 57, 62, 65, 67, 68, 69, 71, 74, 77, 78, 79, 83, 85, 86, 88 |
| 3 | 39 | 6, 7, 8, 18, 19, 20, 21, 22, 23, 24, 28, 29, 30, 32, 34, 36, 38, 39, 41, 46, 48, 49, 50, 55, 56, 58, 59, 60, 61, 63, 64, 66, 72, 73, 75, 76, 81, 84, 87 |
| 4 | 3 | 11, 13, 14 |
| 5 | 3 | 26, 53, 54 |
| 6 | 4 | 31, 70, 80, 89 |
| 7 | 2 | 43, 52 |

Table 5. Averages of eight quantitative variables from seven clusters formed by 89 yam genotypes. Cruz das Almas, BA, Brazil. 2016.

| Variable | Groups | | | | | | |
|----------|--------|-------|-------|-------|-------|-------|-------|
| | G1 | G2 | G3 | G4 | G5 | G6 | G7 |
| SD (mm) | 0.34 | 0.48 | 0.42 | 0.33 | 0.47 | 0.38 | 0.55 |
| PL (cm) | 3.93 | 5.80 | 4.51 | 5.37 | 3.67 | 6.85 | 4.50 |
| P3 (cm) | 4.53 | 4.20 | 3.33 | 7.00 | 2.53 | 5.90 | 2.80 |
| P2 (cm) | 13.62 | 15.39 | 14.11 | 10.97 | 11.80 | 18.33 | 10.90 |
| F1 (cm) | 5.72 | 7.74 | 5.73 | 5.30 | 4.23 | 9.20 | 5.60 |
| TL (cm) | 31.73 | 22.86 | 24.34 | 35.30 | 19.80 | 20.20 | 37.15 |
| TW (cm) | 6.53 | 7.05 | 6.89 | 6.50 | 9.53 | 7.05 | 10.85 |
| WT (kg) | 0.74 | 0.74 | 0.73 | 1.26 | 1.16 | 0.69 | 2.58 |

F1, Leaf width at widest portion; P2 and P3, distance among petiole insertion in the leaf, the lower and upper of the leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter; WT, tuber weight.

These results indicate that there was no relationship between geographic origin and genetic diversity, since genotypes from different municipalities were gathered within same group. This fact is probably due to existence of gene flow resulting from changes of propagative materials among producers of different communities within study area. Similar results were verified by Domingos et al. (2014), Bressan et al. (2014) and Nascimento et al. (2015).

The most dissimilar genotypes, with genetic distance of 0.68 were 31 and 52 (data not shown), being the first from São Félix and the second from São Felipe. Under the agronomic point of view, both genotypes showed the highest values, as for tuber length (35.10 and 39.20 cm), as well as for weight (2.01 and 3.16 kg). The most similar genotypes, with genetic distance of 0.06 were 30 and 32 (data not shown), both belonging to Campo das Flores village, in São Felipe municipality. These genotypes can be considered as clones, since they presented similar values in all analyzed variables. The presence of possible duplicates of *D. alata* L. in the same municipalities was

also observed by Bressan et al. (2011).

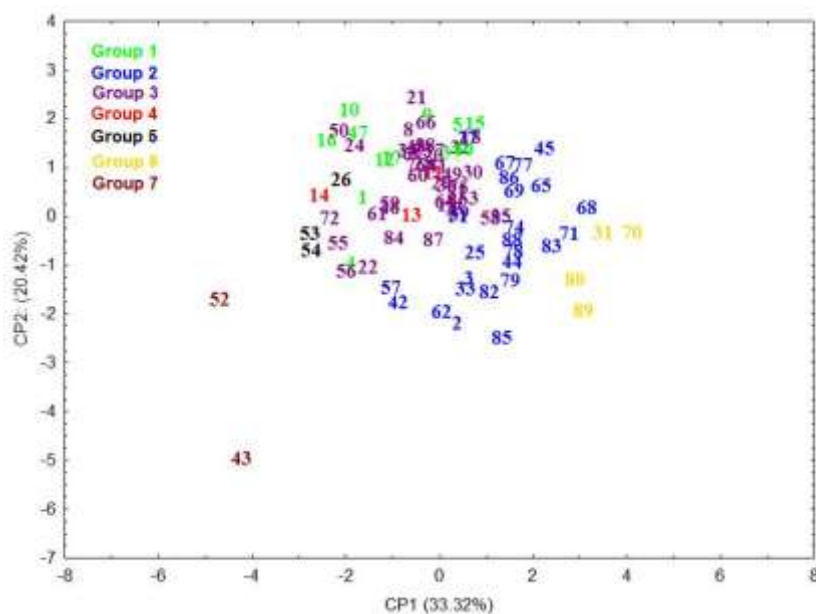
The variables averages of seven groups are shown in Table 5. It is observed that groups 4, 5, and 7, are more promising, due to higher averages for WT, whereas groups 1, 2, 3 and 6 were characterized by smallest averages for WT. Group 6 had the greatest for traits PL, P3, and F1. On the other hand, group 7 showed the greatest traits of SD, TL, TW and WT. As stated by Santos (1996), groups 1, 2, 3, 4, and 5 have tubers with export potential for United States market, while group 7 can be exported to France. Thus, this information is useful in selection of potential genotypes to be used in future genetic improvement programs of yams, aiming to develop cultivars with superior characteristics and meet market demand, as well as provide better incomes for producers in Recôncavo Baiano region, selling quality tubers.

Table 6 presents the estimates of eigenvalues associated with each variable and their total and cumulative variances. It is observed that first three principal components were able to explain only 69.49% of

Table 6. Estimates of eigenvalues associated with each variable and their total and cumulative variances, obtained in the principal component analysis of 89 yam genotypes. Cruz das Almas, BA, Brazil. 2016.

| Variable | Principal components | | | | | | | |
|-------------------------|----------------------|--------|--------|--------|--------|--------|--------|--------|
| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 |
| SD | -0.066 | -0.483 | 0.548 | -0.587 | -0.338 | 0.047 | -0.030 | 0.020 |
| PL | 0.704 | -0.508 | -0.114 | 0.016 | 0.019 | -0.130 | 0.455 | -0.093 |
| P3 | 0.449 | -0.004 | -0.711 | -0.006 | -0.498 | 0.193 | -0.080 | 0.029 |
| P2 | 0.749 | -0.257 | 0.137 | -0.014 | 0.331 | 0.481 | -0.083 | 0.078 |
| F1 | 0.753 | -0.326 | -0.142 | -0.109 | 0.143 | -0.391 | -0.348 | -0.010 |
| TL | -0.527 | -0.090 | -0.560 | -0.533 | 0.287 | 0.091 | 0.004 | -0.163 |
| TW | -0.438 | -0.724 | 0.070 | 0.429 | -0.083 | 0.112 | -0.155 | -0.227 |
| WT | -0.605 | -0.662 | -0.292 | 0.078 | 0.088 | -0.065 | 0.046 | 0.301 |
| Total variance (%) | 33.32 | 20.41 | 15.75 | 10.39 | 7.37 | 5.83 | 4.60 | 2.31 |
| Cumulative variance (%) | 33.32 | 53.73 | 69.49 | 79.89 | 87.26 | 93.08 | 97.69 | 100.00 |

F1, Leaf width at widest portion; P2 and P3, distance among petiole insertion in the leaf, the lower and upper of the leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter; WT, tuber weight.

**Figure 2.** Dispersion graph of 89 yam genotypes based on the first two principal components (CP1 and CP2), according to the groups formed by the UPGMA method.

total variation. The first principal component explained 33.32% of the total variance and was associated with the PL, P2 and F1.

The second and third principal components, with explanation of 20.41 and 15.75% of the total variation, were associated with TW and P3, respectively. These results corroborate with those by Norman et al. (2011) and Muluaem and Michael (2013), in which the first three principal components explained 58 and 65% of total variation, respectively.

According to Cruz et al. (2012), dispersion graph provides satisfactory results when first two principal

components explain 80% of total variation. For this reason, dispersion graph representation of 89 yam genotypes does not only explain variability satisfactorily, since the two first principal components explained only 53.73% of the total variation (Figure 2). The groups formed by dispersion graph were partially consistent with those formed by UPGMA method. Figure 2 shows that there has not been a clear separation of clusters. However, group 7, with only two genotypes, was the most distant from others, corroborating with results obtained in cluster analysis.

The information obtained in this study can contribute to

conservation and genetic improvement of yams in edaphoclimatic conditions from Recôncavo Baiano region. However, molecular characterization of these genotypes has become necessary, to supplement information generated by morpho agronomic descriptors.

Conclusion

There is genetic variability of yams liable to exploitation in programs of conservation and genetic improvement. The tuber length was the variable with most contribution for the genetic divergence.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledges the agreement signed between the Federal University of Recôncavo da Bahia with the Pedagogic University of Mozambique that allowed the graduation of the first author and the Banco do Nordeste by financial support given to this research and the farmers that offered plant material.

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