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Genetic diversity revealed dissimilarity among Mozambican cassava cultivars

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Abstract

The present study aimed to estimate the genetic diversity among 21 cassava genotypes from Mozambique. We also suggested the genotypes with potential to be used as cultivars or in breeding programs based on Mahalanobis distance and agronomic performance. Estimation of relative contribution of each phenotypic trait to genetic diversity was also carried out. Genotypes were evaluated using eight phenotypic traits in an experiment at Mogincual district, Mozambique. The experiment consisted of a randomized block design with 21 genotypes with three replications. The plant height, first branch height, shoot biomass yield, mean number of tuberous roots per plant, tuberous roots yield, production of commercial roots, harvest index and dry matter content were evaluated in the analyses. The obtained data were subjected to analysis of variance and to Scott and Knott ($p \leq 0.05$). Genetic diversity was expressed by the generalized Mahalanobis distance with subsequent clustering of genotypes by the Tocher's analysis, UPGMA and graphical dispersion. Graphical dispersion was used to illustrate differences between genotypes and to verify the agreement of the results obtained these different clustering methods. The results showed that Tocher and UPGMA formed 4 groups and genotypes were allocated in each group for both methods. The relative contribution of the traits for diversity was based on the method of Singh. There are genetic differences among the studied genotypes. The genotypes MzMg10/096, MzMg10/630, MzMg10/240, MzMg10/314 and MzMg10/162 are potentially useful to participate in breeding programs because they are divergent with high mean for the evaluated traits. Shoot biomass weight (48.10%) and number of roots per plant (18.40%) were the most important traits for genotypes discrimination.

Keywords: biometric analyses; diversity; Genetic breeding; *Manihot esculenta* crantz; statistics.
Abbreviations: ANOVA_analysis of variance; D^2ii'_Mahalanobis distance; DMC_dry matter content; FBH_first branch height; HI_ harvest index; IIAM_Agricultural Research Institute of Mozambique; NRP_mean number of roots per plant; PCR_production of commercial roots; PH_plant height; RY_tuberous roots yield; SBY_shoot biomass yield.

Introduction

Cassava (*Manihot esculenta* Crantz.) belongs to the Euphorbiaceae family, which includes approximately 6,300 species (Hurdack et al., 2005). Botanically, it is a tropical perennial shrub whose origin center is the Amazon Basin (Olsen et al., 1999). Cassava typically is a diploid species (*2n=2x=36*) (Raji et al., 2009; Sakurai et al., 2013), highly heterozygous due to its vegetative propagation through stakes in agriculture. Cassava is cultivated in more than 100 countries and its leaves and roots can be consumed as food and feed (Taylor et al., 2012). Cassava is of great social importance and is one of the most important sources of carbohydrates in the tropics. It is being consumed as food, animal feeding and processing industry by approximately 700 million people worldwide (FAO, 2009). This plant is cultivated all over Mozambique, with a consequent diversity of cultivars adapted to each different biome throughout the country, giving the species great genetic diversity (Zacharias and Cuambe, 2004). Cassava is characterized by its wide genetic diversity, which in turn, generates a multitude of individuals able to adapt to different eco-geographic growing regions. Fukuda and Silva, (2002) report that the great genetic diversity presented in cassava is a result of natural selection during the evolution and domestication of the species. The species has a high cross-pollination potential and has high heterozygosity, which continuously produces several new genotypes. Genetic diversity is important since the success of any breeding program is based on the presence of variability for the desirable traits. In this context, genetic diversity is essential for breeding programs. For breeders, two parameters are essential: high means for each individual in breeding population and variability between and within genotypes. Such studies can help to identify phenotypic differences among individuals within a particular population. So, the potential and aptitude of each individual are described (Araújo, 2002), providing the breeders useful information for the selection of genotypes that address the needs of breeding programs (Mezetti et al., 2013; Rimoldi et al., 2010). Genetic diversity can be estimated by evaluation of similarity or dissimilarity index of genotypes and use of biometric techniques. The estimation may be carried out in two different ways: quantification of heterosis in studies involving diallel analysis, or by the use of predictive methods

based on phenotypic and genotypic data, which are obtained by observing morphological, physiological or molecular differences. After quantification, genetic diversity can be demonstrated using multivariate statistical techniques (Cruz et al., 2011; Cruz et al., 2012), such as the generalized Mahalanobis distance, the canonical variables and the principal components, etc. The method choice depends on the desired accuracy, easiness of analysis and on the manner of obtaining data. These studies may be complemented by the Tocher's analysis and by dispersion graph, which employs previously estimated matrices of genetic distances (Cruz et al., 2011).

Agronomic traits of cassava clones have been used for determining the diversity to select materials that can be improved as cultivars, or used in future breeding programs (Nick et al., 2010). During the selection of genotypes for participating in a breeding program, it is required to implement the phenotyping of individuals. The use of quantitative agronomic traits is indispensable, since the main assumption for crossing prediction based on biodiversity estimates is that, besides diversity, the agronomic performance is considered (Mezette et al., 2013).

Several studies have reported morphological characterization to determine the genetic diversity among cassava genotypes in other countries (Benesi et al., 2010; Rimoldi et al., 2010; Asare et al., 2011). However, there is not sufficient studies involving local and improved cassava cultivars neither in Mozambique, nor on the identification of cultivars that could be used in future breeding programs, and description of traits that contribute to genotypic diversity (Kawuki et al., 2013; Mezette et al., 2013; Pariyo et al., 2013).

This study involved the commercial and local cassava cultivars and found high diversity in the population. The objectives of this study were to estimate the genetic diversities among 21 cassava genotypes from Mozambique, based on agronomic traits. We also estimated the relative contribution of each trait for diversity and suggested genotypes with high potential to be used as cultivars or in breeding programs, based on dissimilarity and on agronomic performance.

Results and discussion

Statistical analysis

Prior to analysis of variance (ANOVA), data were submitted to normality test, by the Lilliefors test, which indicated that it is reasonable to study the data through normal distribution ($p \leq 0.05$). ANOVA data presented significant differences by the F-test ($p \leq 0.01$) for all the phenotypic traits evaluated. Thus, the different behavior of genotypes portrays the presence of variability for the agronomic traits studied. The presence of wide variability was expected, since it was evaluated genetic constitutions of different origins and improvement levels, as previously reported by Borges et al. (2002) and Nick et al. (2008).

The existence of high variability in cassava among genotypes derived from the genetic improvement may be explained by the broad genetic base available in germplasm banks in Mozambique, which is used constantly in breeding programs. On the other hand, it may also be explained by the fact that the species is allogamous (El-Sharkawy, 2004), allowing new combinations to be naturally and constantly generated. These combinations are initially propagated by seeds, and then they are vegetatively propagated, just like a new cultivar (Ceballos et al., 2004).

Genetic diversity

Genetic diversity, estimated by the Mahalanobis distance (D^2ii'), showed that the combination between genotypes MzMg10/162 and Tomo was the most dissimilar for presenting the maximum distance, since the D^2ii' value was 303.15. Meanwhile, the lowest magnitude distance was presented by the combination of genotypes MzMg10/083 and MgMz10/107, since the D^2ii' value was 5.23, and they were the most similar genotypes, showing genetic proximity by virtue of the evaluated traits (Distance Matrix not shown).

The use of diversity measures for the choice of parents has allowed identifying hybrid combinations superior to the parents. However, it is important to note that the use of more divergent individuals as parents does not necessarily imply obtaining heterosis, as the heterosis may occur only in a few hybrids. Borém and Miranda (2009) point out that, in addition to genetic diversity, the parents' *per se* performance as well as the allelic complementarity between them should be considered, when choosing the parents for breeding programs and selecting the superior individuals in segregating generations.

According to this criterion, the analysis of agronomic performance of MzMg10/162 clone indicates that it is the one with the best performance for presenting high shoot biomass yield (49.08 t ha^{-1}), mean number of tuberous roots per plant (11.57), and especially for having yield of tuberous roots (44.63 t ha^{-1}). However, the "landrace" Tomo does not present satisfactory agronomic development, mainly for the mean number of tuberous roots (1.24), and for the yield of tuberous roots (6.04 t ha^{-1}) (Table 1). Thus, despite these genotypes being the most divergent, the low performance of "landrace" Tomo results in progenies with low agronomic performance, because this genotype is low yielding and susceptible to many diseases.

Clustering analysis

Application of multivariate analysis such as the Tocher method facilitates the interpretation of the joint data. Thus, it is possible to suggest crossings between individuals belonging to different groups and to analyze the group mean for the agronomic trait desired to be improved.

The use of Tocher method, based on the similarity matrix, expressed by the generalized Mahalanobis distance (D^2ii') has allowed the division of the 21 genotypes in six genetically distinct clusters (Table 2). Cluster I included 13 genetically similar genotypes (61.90%), indicating that possible crossings between these genotypes theoretically reduces superior materials. The second, third and fourth clusters were formed by 2 genotypes, and the other groups were formed by one genotype each, which is in agreement with the dissimilarity values presented by Mahalanobis. According to Vieira et al. (2005), clusters formed by only one

Table 1. Scott & Knott test of eight agronomic traits evaluated in twenty one cassava genotypes.

Genotypes	PH	FBH	SBY	NRP	RY	PCR	HI	DMC
	---------m---------		t ha^{-1}	n.^{o}	-------t ha^{-1}-------		----------%----------	
MzMg10/040	2.31 ^{a}	1.10 ^{b}	17.41 ^{d}	7.74 ^{b}	34.17 ^{b}	21.57 ^{b}	65.24 ^{a}	36.50 ^{a}
MzMg10/042	2.20 ^{a}	0.83 ^{c}	14.81 ^{d}	1.13 ^{c}	15.56 ^{c}	13.15 ^{c}	51.51 ^{b}	31.50 ^{a}
MzMg10/075	2.20 ^{a}	1.23 ^{b}	17.59 ^{d}	5.89 ^{b}	27.96 ^{b}	20.00 ^{b}	61.11 ^{a}	21.85 ^{b}
MzMg10/083	2.22 ^{a}	1.67 ^{a}	25.00 ^{c}	6.41 ^{b}	34.81 ^{b}	26.67 ^{a}	58.53 ^{a}	34.48 ^{a}
MzMg10/096	2.60 ^{a}	1.76 ^{a}	46.30 ^{a}	8.93 ^{a}	42.96 ^{a}	34.08 ^{a}	47.81 ^{b}	31.67 ^{a}
MzMg10/107	2.25 ^{a}	1.74 ^{a}	19.26 ^{d}	6.89 ^{b}	30.56 ^{b}	20.37 ^{b}	59.38 ^{a}	27.23 ^{a}
MzMg10/111	2.24 ^{a}	1.74 ^{a}	14.63 ^{d}	9.67 ^{a}	37.74 ^{b}	21.48 ^{b}	69.17 ^{a}	33.53 ^{a}
MzMg10/118	2.07 ^{a}	1.13 ^{b}	19.07 ^{d}	6.70 ^{b}	41.48 ^{a}	30.37 ^{a}	68.20 ^{a}	31.13 ^{a}
MzMg10/129	2.40 ^{a}	0.98 ^{b}	30.93 ^{b}	7.11 ^{b}	37.74 ^{b}	22.59 ^{b}	53.89 ^{b}	27.81 ^{a}
MzMg10/131	1.83 ^{b}	1.17 ^{b}	7.22 ^{e}	1.93 ^{c}	10.74 ^{c}	5.93 ^{c}	59.94 ^{a}	16.51 ^{b}
MzMg10/162	2.22 ^{a}	1.22 ^{b}	49.08 ^{a}	11.57 ^{a}	44.63 ^{a}	18.89 ^{b}	47.19 ^{b}	19.17 ^{b}
MzMg10/168	2.18 ^{a}	1.33 ^{b}	26.11 ^{c}	7.56 ^{b}	43.89 ^{a}	37.74 ^{a}	63.03 ^{a}	22.33 ^{b}
MzMg10/240	1.80 ^{b}	0.73 ^{c}	33.89 ^{b}	6.07 ^{b}	31.48 ^{b}	20.00 ^{b}	47.89 ^{b}	39.95 ^{a}
MzMg10/314	1.92 ^{b}	0.40 ^{d}	36.30 ^{b}	9.87 ^{a}	38.52 ^{b}	21.85 ^{b}	51.19 ^{b}	28.60 ^{a}
MzMg10/354	2.35 ^{a}	1.44 ^{b}	6.48 ^{e}	1.19 ^{c}	10.00 ^{c}	8.15 ^{c}	57.92 ^{a}	29.03 ^{a}
MzMg10/466	1.95 ^{b}	0.67 ^{c}	8.71 ^{e}	2.04 ^{c}	13.33 ^{c}	11.30 ^{c}	60.01 ^{a}	29.78 ^{a}
MzMg10/630	2.33 ^{a}	1.20 ^{b}	44.81 ^{a}	10.41 ^{a}	52.22 ^{a}	41.85 ^{a}	53.83 ^{b}	28.75 ^{a}
Mokhalana	2.32 ^{a}	1.32 ^{b}	13.52 ^{d}	4.63 ^{b}	20.37 ^{c}	15.93 ^{b}	59.98 ^{a}	37.37 ^{a}
Orera	1.67 ^{c}	0.43 ^{d}	9.07 ^{e}	4.00 ^{c}	17.04 ^{c}	13.71 ^{c}	64.64 ^{a}	31.59 ^{a}
Tomo	1.98 ^{b}	1.78 ^{a}	5.74 ^{e}	1.24 ^{c}	6.04 ^{c}	3.70 ^{c}	53.41 ^{b}	21.67 ^{b}
Varuiaya	1.53 ^{c}	0.87 ^{c}	8.70 ^{e}	5.61 ^{b}	34.63 ^{b}	18.52 ^{b}	80.03 ^{a}	27.97 ^{a}
General Mean	2.14	1.18	21.65	6.03	29.61	20.28	58.76	28.83
Range$^{(1)}$	1.07	1.38	43.34	10.44	46.18	38.15	32.84	20.86

* Means group followed by the same letter in the column do not differ by the Scott & Knott (1974) test, modified by Bhering et al. (2008), at 5% probability. $^{(1)}$ Difference between the highest and the lowest mean. PH - Plant height, FBH - first branch height, SBY - shoot biomass, NRP - mean number of roots per plant, RY - roots yield, PCR - production of commercial roots, HI - harvest index, DMC - dry matter content.

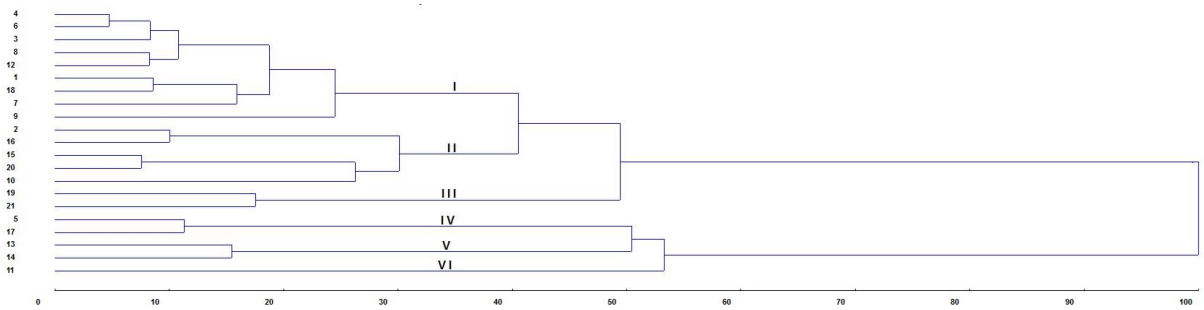

Fig 1. Illustrative Dendrogram of dissimilarity standard established by UPGMA, based on eight agronomic traits among 21 cassava genotypes: 1 = MzMg10/040; 2 = MzMg10/042; 3 = MzMg10/075; 4 = MzMg10/083; 5 = MzMg10/096; 6 = Mg10/107; 7 = MzMg10/111; 8 = MzMg10/118; 9 = MzMg10/119; 10 = MzMg10/131; 11 = MzMg10/162 12 = MzMg10/168; 13 = MzMg10/240; 14 = MzMg10/314; 15 = MzMg10/354; 16 = MzMg10/466; 17 = MzMg10/630; 18 = Mokhalana; 19 = Orera; 20 = Tomo; 21 = Varuiaya.

individual indicate that these individuals are more divergent than the others. With regard to cluster analysis by UPGMA, Fig 1 shows the representative dendrogram of dissimilarity among the evaluated genotypes. The value of cophenetic correlation was 0.69. In previous studies, the cophenetic correlation coefficients with values above 0.60 were reported (Busato et al., 2004; Vieira et al., 2005). According to Vaz Patto et al. (2004), r values $\geq$ 0.56 are considered ideal, indicating that the dendrogram reproduces satisfactorily the information contained in the correlation matrix, and in the consequent cluster formation.

When subjected to a vertical cut at a distance of about 40% dissimilarity, UPGMA indicated the formation of six clusters. Cluster I was formed by a large number of genotypes, a total of 9 out of 21 genotypes analyzed. Cluster II consisted of five genotypes, and the other groups were composed of two genotypes, with the exception of the last cluster (V), represented by only one genotype.

Tocher method (Table 2) and UPGMA (Fig 1) showed a trend to discriminate genotypes by clusters in a similar way. UPGMA also had the highest portion of genotypes allocated in cluster I, and presented cluster formation consisting of one genotype. It can be observed that the genotypes which formed clusters II, III and IV by the Tocher method were the same genotypes which formed clusters IV, V and III by the UPGMA, suggesting that the methods are in agreement in clustering, but differing sometimes in the order. Another point to be considered is the fact that MzMg10/162 genotype forms the last cluster by itself in both methods. The UPGMA offered a more detailed cluster, which allowed visualizing distances within a particular cluster, complementing the Tocher's analysis, which in turn, provided different clusters. This was confirmed by Kvitschal (2008), while studying the characterization and genetic diversity of cassava germplasm of Maringa region, who concluded that the combination of

Table 2. Representation of clustering of twenty-one cassava genotypes generated by the Tocher method, based on dissimilarity expressed by the generalized Mahalanobis distance, for eight agronomic traits [(1)].

Clusters	Genotypes/Cultivars
I	MzMg10/083, MzMg10/107, MzMg10/075, MzMg10/168, MzMg10/118, Mokhalana, MzMg10/040, MzMg10/111, MzMg10/119, MzMg10/466, MzMg10/042, MzMg10/354, MzMg10/131
II	MzMg10/096, MzMg10/630
III	MzMg10/240, MzMg10/314
IV	Orera, Varuiaya
V	Tomo
VI	MzMg10/162

[(1)] PH - plant height, FBH - first branch height, SBY - shoot biomass yield, NRP - mean number of roots per plant, PCR - production of commercial roots, HI- harvest index, DMC - dry matter content, RY - roots yield.

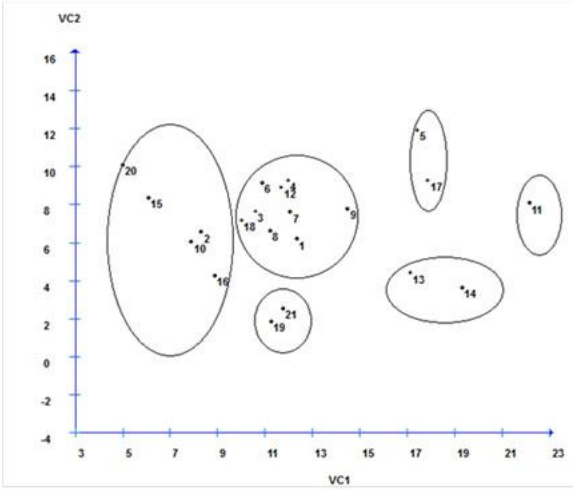

Fig 2. Graphical dispersion of scores for the two representative axes of the first two canonical variables (CV_1 e CV_2) for eight agronomic traits in 21 cassava genotypes: 1 = MzMg10/040; 2 = MzMg10/042; 3 = MzMg10/075; 4 = MzMg10/083; 5 = MzMg10/096; 6 = Mg10/107; 7 = MzMg10/111; 8 = MzMg10/118; 9 = MzMg10/119; 10 = MzMg10/131; 11 = MzMg10/162 12 = MzMg10/168; 13 = MzMg10/240; 14 = MzMg10/314; 15 = MzMg10/354; 16 = MzMg10/466; 17 = MzMg10/630; 18 = Mokhalana; 19 = Orera; 20 = Tomo; 21 = Varuiaya.

Table 3. Means of discrimination groups of cassava genotypes by the Tocher's analysis, regarding eight agronomic traits.

Phenotypic Traits	Means of clusters (*) formed by the Tocher's analysis					
	I (*)	II (*)	III (*)	IV (*)	V (*)	VI (*)
PH	2.19	2.47	1.86	1.60	1.98	2.22
FBH	1.26	1.48	0.57	0.68	1.78	1.22
SBY	16.98	45.56	35.10	8.89	5.74	49.08
NRP	5.30	9.67	7.97	4.81	1.24	11.57
RY	27.57	47.59	35.00	25.84	6.04	44.63
PCR	19.63	37.97	20.93	16.12	3.70	18.89
HI	60.61	50.82	49.54	72.34	53.41	47.19
DMC	29.16	30.21	34.28	29.78	21.67	19.17

PH - plant height, FBH - first branch height, SBY - shoot biomass yield, NRP - mean number of roots per plant, PCR - production of commercial roots, HI- harvest index, DMC - dry matter content, RY - roots yield.

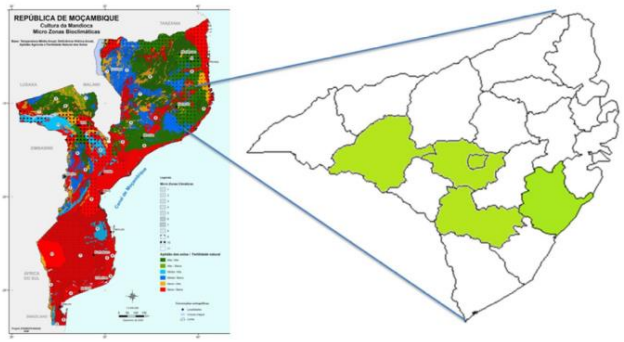

Fig 3. Map of Mozambique showing Nampula province, where the experiments were carried out and samples were collected.

both methods allows a better orientation in cassava breeding. The similarity in the discrimination of genotypes for genetic diversity between these two methods has also been reported in cassava (*M. esculenta* Crantz) (Kamuki et al 2013; Njoku et al 2013), pumpkin (*Curcubita maxima*) (Gobena et al. 2012), common beans (*Phaseolus vulgaris*) (Sharma et al. 2013) and Jatropha (Bhering et al, 2013).

The genotypes located in more distant clusters are inferred to be dissimilar. They can be considered promising in artificial crossings, i.e. these genotypes can be used in diallel crossing and generate hybrid with high performance. However, when choosing the parents for a breeding program, one should consider their "*per se*" potential (Souza et al, 2005; Borém and Miranda, 2009).

Scott & Knott method, modified by Bhering et al. (2008) (Table 1) showed superior genotypes based on in their means, while Tocher and UPGMA showed how these genotypes were distributed between groups. These analyses together allowed the identification of the promising crosses to recognize which one may result in restricted variability in segregating generations, such as those carried out between parents of the same cluster. In this sense, depending on the objectives of the breeding program, some crossings are suggested. For the reduction of cassava plant architecture with no yield loss, crossings between individuals of clusters III and IV (Tables 2 and 3) are suggested. These groups showed the lowest means for plant height, which did not compromise yield. On the other hand, crossings between individuals of clusters II and VI could be promising if the program aims to obtain plants with larger size and higher yield. Crossings between individuals of clusters II and VI may also be promising if the objective is to obtain plants with higher shoot biomass yield, with no loss in root yield.

For genotypes with high yield of tuberous root and dry matter, it is suggested crossings between individuals of clusters II and III, since these clusters had higher means for root yield, as well as higher percentage of dry matter (Tables 2 and 3).

Selecting superior genotypes

For the initial selection of individuals based on agronomic performance of mean number of tuberous roots per plant and tuberous roots yield per hectare, it is suggested crossings between individuals of clusters II, III and VI (Tables 2 and 3). The individual performance of each component of the clusters (Table 3) reinforces this expectation. From the means analyses of MzMg10/096 and MzMg10/630 genotypes (Cluster II), MzMg10/240 and MzMg10/314 genotypes (Cluster III), and MzMg10/162 genotype (Cluster VI), it is concluded that the *per se* performance of these genotypes is satisfactory.

Table 4. lists the eigenvalues and the coefficients associated with the variables analyzed, expressing their relative importance in the genetic diversity study. Variables with little variability, or those are correlated with other variabilities, taken into account in the present study had coefficients of great magnitude in the last eigenvectors. Considering the last canonical variables, which represent less than 5% of the total variation, it is observed that harvest index (HI), production of commercial roots (PCR), dry matter content (DMC) and mean number of roots per plant (NRP) could be discarded for future studies.

The use of score projections in 2D Cartesian plane in the genetic diversity study aims to identify more dissimilar genotypes in two-dimensional scatter plots, seeking to simplify results interpretation (Cruz et al. 2011).

According to Cruz et al (2012), researchers have opted for graphical representation, when the first two canonical variables have about 80% of the total variation. Only when this limit is not reached in the first two components, the analysis is complemented by the graphic dispersion in relation to the third component. However, in this study, the analysis revealed that the first two canonical variables accounted for about 78.02% of the total variation (58.27% for the first, and 19.75% for the second) (Table 4), which is satisfactory in this case. However, this analysis did not explain the genetic diversity by graphical dispersion of about 80% of the total variance, in relation to axes represented by these two canonical variables, with a negligible degree of distortion caused by the distance between genotypes.

As mentioned before, the results of clustering analysis are of great importance in planning breeding programs to obtain heterotic hybrids and base population for future programs, as they help indicating clusters and/or distinct sub-clusters to be included in these selection programs.

The graphical dispersion analysis, based on the first two canonical variables (Fig 2) allows the formation of six clusters quite similar to those obtained by clustering methods (Table 2 and Fig 1). It was observed that Tomo and MzMg10/162 were the most distant genotypes in relation to the others, while MzMg10/083 and MzMg10/107 were the closest. Thus, they are reliable in the identification of parents with high diversity to point out promising crossings.

Recent studies have shown that quantitative traits are essential for the evaluation of genetic diversity and genetic improvement. Therefore, in this study it was found that the quantitative morphological and agronomic traits are viable tool in the evaluation of genetic diversity between cassava genotypes, mainly when field experiment has many replications, where the heritability is high, and the environment effect is low.

The traits of minor importance for genetic diversity between the genotypes clusters were those that weighting coefficients obtained by variables standardization in the last canonical variables are of greater magnitude in absolute value, as they are responsible for minimum fraction of the total variance available (Cruz et al., 2011).

Table 5. shows the relative contribution of each agronomic trait for genetic dissimilarity, according to the Singh (1981) method. Results indicate that the two traits with greater contribution to the genetic diversity were shoot biomass yield (48.77%) and mean number of roots per plant (16.93%). This suggests that these traits are efficient in explaining the dissimilarity between genotypes and they may be prioritized in the choice of materials for crossing purposes. Similar results were also observed in works carried out by Nick et al. (2010), who studied the genetic diversity between cassava subsamples in Lavras, Minas Gerais. Among the evaluated agronomic traits, shoot biomass yield and mean number of roots per plant were the most important traits in explaining the genetic diversity.

Gomes et al. (2007) reported that traits such as shoot biomass yield and mean number of tuberous roots per plant are essential in cassava breeding programs, since they can be used as auxiliary selection criteria for tuberous root yield.

According to the Singh (1981) method, traits with less variability, or those are represented by other traits, are considered of less importance. In this study, the traits that presented the lowest variability were harvest index, dry matter content in roots, and production of commercial roots, respectively.

Table 4. Accumulated eigenvalues corresponding to percentages of variation, explained by the canonical variables (CV), and weighting coefficients (eigenvectors) of eight agronomic traits evaluated in twenty one cassava genotypes evaluated in Agricultural Research Institute of Mozambique (IIAM) in the district of Mogincual, Nampula province, located in the North of Mozambique.

VC	Accumulated eigenvalues	Weighting coefficients associated to:							
		PH	ALTPR	SBY	NRP	RY	PCR	HI	DMC
VC_1	58.27	-0.300	-0.244	1.596	0.956	-0.803	-0.703	1.239	0.342
VC_2	78.02	0.505	0.703	0.059	-0.390	0.190	0.389	-0.445	-0.345
VC_3	86.06	-0.621	0.660	0.443	0.525	-0.652	-0.622	0.943	-0.520
VC_4	92.06	-0.592	0.407	-0.025	-0.359	-0.052	1.058	0.194	0.154
VC_5	96.55	0.225	0.052	-0.317	0.899	-0.196	-0.307	0.071	0.681
VC_6	98.21	0.294	-0.335	-0.812	0.188	1.268	-0.078	-1.013	-1.450
VC_7	99.38	0.073	0.066	-0.186	-0.942	0.307	-1.194	-0.395	0.204
VC_8	100.00	0.329	-0.132	0.803	-0.235	-0.937	-0.087	1.802	-0.028

PH - plant height, FBH - first branch height, SBY - shoot biomass yield, NRP - mean number of roots per plant, PCR - production of commercial roots, HI- harvest index, DMC - dry matter content, RY - roots yield.

Table 5. Relative contribution of eight agronomic traits for genetic diversity of twenty-one cassava genotypes, using the Singh (1981) method.

Agronomic traits	Relative Importance (%)
PH	7.23
FBH	10.42
SBY	48.77
NRP	16.93
RY	8.97
PCR	0.99
HI	2.74
DMC	3.97

PH - plant height, FBH - first branch height, SBY - shoot biomass yield, NRP - mean number of roots per plant, PCR - production of commercial roots, HI- harvest index, DMC - dry matter content, RY - roots yield.

Table 6. Identification list and pedigree of 21 cassava genotypes used for the study.

Varieties	Pedigree	Origin	Special atributes
Mokhalana	PAN 38 (OP)	IIAM Improved Variety	CBSD resistance, higy yield
MzMg10/040	Mulaleia x IMM30025	IIAM Improved clone	CBSD and CMD resistance, high dray matter content
MzMg10/042	Likonde x Mulaleia	IIAM Improved clone	CBSD and CMD resistance, higy dry matter content
MzMg10/075	Mulaleia (OP)	IIAM Improved clone	CBSD and CMD resistance, higy dry matter content
MzMg10/083	Likonde x Chigoma máfia	IIAM Improved clone	Higy yield, CBSD and CMD resistance
MzMg10/096	Likonde x Mulaleia	IIAM Improved clone	Higy yield, good plant type, good leaves for consumption
MzMg10/107	Likonde x Mulaleia	IIAM Improved clone	Higy yield, good plant type, good harvest index
MzMg10/111	Mulaleia (OP)	IIAM Improved clone	Higy yield, good plant type
MzMg10/118	Mulaleia (OP)	IIAM Improved clone	CBSD tolerance, CMD resistance, higy dry matter content
MzMg10/129	Mulaleia x Likonde	IIAM Improved clone	Higy yield, good plant type
MzMg10/131	Mulaleia (OP)	IIAM Improved clone	CBSD and CMD resistance, Higy yield, good type of plant
MzMg10/162	Mulaleia x Likonde	IIAM Improved clone	Higy yield, good plant type, good harvest index
MzMg10/168	Mulaleia x Chigoma máfia	IIAM Improved clone	Higy yield, good plant type, higy dry matter content
MzMg10/240	Mulaleia x Chigoma máfia	IIAM Improved clone	Higy yield, CBSD resistance, higy dray matter content
MzMg10/314	Mulaleia (OP)	IIAM Improved clone	Higy yield, CBSD and CMD resistance
MzMg10/354	Mulaleia x Likonde	IIAM Improved clone	CBSD tolerance, CMD resistance, higy dry matter content
MzMg10/466	Mulaleia x Likonde	IIAM Improved clone	CBSD tolerance, CMD resistance, higy dry matter content
MzMg10/630	Mulaleia x TMS30001	IIAM Improved clone	Higy yield, CBSD and CMD resistance
Orera	Likonde (OP)	IIAM Improved variety	CBSD and CMD resistance, good leaves for consumption
Tomo	Tomo	Landrance	Good type of plant, good leaves for consumption
Varuiaya	IMM30025 X Chigoma máfia	IIAM Improved Variety	CBSD Tolerance, higy dry matter content

OP = Open pollination, CBSD = Cassava Brown Streak Disease, CMD = Cassava Mosaic Disease, IIAM= Agricultural Research Institute of Mozambique.

Therefore, after analyzing the results of the relative importance of traits for genetic diversity by Singh (1981) methodology, and by the canonical variables technique, it is observed that both methods identified the traits that little contributed for diversity, although they are not identical in the discarding order.

Materials and Methods

Plant materials

Twenty-one cassava genotypes from Mozambique were chosen for this study. A randomized block design in three replications was used as experimental design and twenty-one cassava genotypes were planted as treatments. The genotypes were selected for their contrasting agronomic performance, and also for being preferred by cassava farmers in northern region of Mozambique. The genotypes used in the experiment are shown in Table 6. Fig 3 also shows the location where each genotype was collect.

Experimental design

The experiment was carried in a randomized block design, with twenty-one genotypes planted in three replications. The experimental plots consisted of five rows with five plants each, spaced 1.0 m between rows, and 1.0 m between plants. The useful plot area consisted of three rows and three central plants, covering an area of 9.0 m^2, consisting of 9 plants per plot. The external lines were considered as borders.

The experiment was carried out in the experimental field of the Agricultural Research Institute of Mozambique (IIAM) in the district of Mogincual, Nampula province, located in the North of Mozambique.

The climate was Cwa type (humid temperate climate with dry winter and hot summer), according to the Köppen classification. The district had an average annual relative humidity of 80%, an average annual temperature of 26^o C (ranging from 17° C to 30^o C the minimum and maximum averages, respectively), and average annual rainfall ranging from 800 to 1,000 mm, of which 65-70% of it concentrated in the period from December to March.

In this region, the soils are predominantly sandy, brown-grayish, deep, and with good drainage capacity. However, they are moderately acidic with low organic matter (MAE, 2005).

The experiment was carried out in non-irrigation regime during the 2011/12 growing season. Soil tillage consisted of plowing and harrowing, as the most common agricultural practices among the regional producers. There were no furrows, and planting was carried out in shallow soil.

The selection of cuttings for planting was carried out seeking to standardize the materials used. The cuttings (with an average size between 20 cm and 25 cm in length, and about two centimeters in diameter, and five to seven nodes) were taken from the middle third of healthy plants with 12 months of age, which were planted vertically in the soil, at approximately 16 cm depth. A straight cut was carried out in both ends in order to obtain the cuttings.

Cuttings were planted in December, at the beginning of the rainy season, which is planting season recommended for the district of Mogincual. This region is characterized by periods of short rain from December to March.

The experiment and all cultural practices were carried out according to the recommendations of cassava production system for this coastal region of northern Mozambique. During the experiment, weed control was carried out manually with hoe to prevent competition. Neither traditional nor topdressing manuring were carried to allow the experiment simulate the conditions similar to those prevailing in the fields of local cassava producers.

At harvest, which was carried out 12 months after the emergence of plants, phenotypic data from eight quantitative traits were collected as following: plant height (PH) (meters); first branch height (FBH) (meters); shoot biomass yield (SBY) (t ha^{-1}); mean number of tuberous roots per plant (NRP); tuberous roots yield (RY) (t ha^{-1}); production of commercial roots (PCR) (t ha^{-1}); harvest index (HI) (%); and dry matter content (DMC) (%).

Statistics analysis

Once statistical difference between treatments (genotypes) by analysis of variance (ANOVA) verified, Scott and Knott (1974) test, modified by Bhering et al. (2008) was carried out the, at 5% probability. The analysis of variance was used because there was no missing data. Therefore, it was not necessary to use Best Linear Unbiased Prediction (BLUP).
The genetic diversity among genotypes was evaluated by the Tocher method and UPGMA, having the generalized Mahalanobis distance ($D^2_{ii'}$) as a measurement (Cruz, et al., 2011). The Mahalanobis distance was used because there were many replications for each genotype. All variables were standardized.

The analysis based on canonical variables technique was also used to study genetic diversity among cassava genotypes to better illustrate these differences and to verify the accordance of the results obtained by these different analysis methods, which allows more reliable interpretation of the results. The relative importance of traits in relation to genetic diversity among genotypes was studied by the method proposed by Singh (1981). This method is based on Mahalanobis distance. Statistical analysis of experimental data was carried out using the GENES software (Cruz, 2013).

Conclusion

A valuable genetic diversity was observed among the studied genotypes. The genotypes MzMg10/096, MzMg10/630, MzMg10/240, MzMg10/314 and MzMg10/162 are potentially useful to participate in future stages of a breeding program in Mozambique and can also be used in other countries. The traits that most contributed to the genetic diversity were shoot biomass yield and number of roots per plant. On the other hand, the traits that contributed least were production of commercial roots, harvest index, and dry matter in tuberous roots. These results will guide the selection of genotypes in cassava breeding programs as a subsidy in the knowledge of the degree of genetic variability available between the genotypes evaluated.

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